PB-216 919

BIOLOGY OF WATER POLLUTION. A COLLECTION OF SELECTED PAPERS ON STREAM POLLUTION, WASTE WATER, AND WATER TREATMENT

Lowell E. Keup, et al

Federal Water Pollution Control Administration
Cincinnati, Ohio

1 October 1967

DISTRIBUTED BY:

NTIS
National Technical Information Service
U. S. DEPARTMENT OF COMMERCE
5285 Port Royal Road, Springfield Va. 22151

PB 216 919

BIOLOGY OF WATER POLLUTION

A Collection of Selected Papers on Stream Pollution, Waste Water, and Water Treatment

Compiled
by

Lowell E. Keup
William Marcus Ingram
Kenneth M. Mackenthun

Second Printing - June 1968
Third Printing - May 1970

PRICES SUBJECT TO CHANGE

Reproduced by
NATIONAL TECHNICAL
INFORMATION SERVICE
U S Department of Commerce
Springfield VA 22151

Details of illustrations in
this document may be better
studied on microfiche

PRICES SUBJECT TO CHANGE

UNITED STATES DEPARTMENT OF THE INTERIOR
Federal Water Pollution Control Administration
1967

. 298

PREFACE

Water users are becoming more and more concerned with the abatement of pollution. Accelerated population and industrial growth has brought many persons into intimate contact with problems relating to water degradation that is associated with municipal, industrial, or agricultural wastes, or combinations of these. Thus, the problems of waste disposal and their reasonable solution are today the concern of all. It is apparent that problems attendant to waste disposal and water treatment are increasing. Most people fully appreciate that streams, lakes, and estuarine waters remain static in quantity.

With increased concern for America's water resources, the populace is demanding accelerated programs for the abatement of pollution, and this requires an increased number of personnel trained in and cognizant of environmental science.

Science is common sense based upon the experiences of man. Scientific literature is a record of these experiences. With the growth of the aquatic sciences, the literature has become voluminous, creating an information retrieval problem. In addition, many of the earlier writings were published in limited editions and some in not readily available journals. Today many are not readily available to the scientist who does not have access to a large, long established library.

This book of selected publications on Biology of Water Pollution, Water Treatment, and Sewage and Industrial Waste Treatment contains some of the many excellent and basic pertinent biological papers that have been commonly inaccessible to the contemporary investigator. These papers are often quoted (sometimes incorrectly) and are a portion of the "foundation" upon which modern aquatic ecological scientific thought and decisions are often based in summating water pollution control investigations.

This compiled collection will be of assistance in three phases of water pollution abatement: (1) It will provide a technical service to the aquatic ecologist through the assemblage of informative literature; (2) it will illustrate many of the concepts upon which regulations have been formulated for the protection of aquatic life; (3) it will aid in the training of new environmental scientists to meet today's and tomorrow's personnel needs in the conservation of our Nation's natural resources.

Lowell E. Keup
William Marcus Ingram
Kenneth M. Mackenthun

Cincinnati, Ohio
October 1, 1967

ACKNOWLEDGMENTS

The compilers are indebted to all the authors of the articles incorporated in this publication. With their permission to reproduce their work, we have the opportunity to present a collection of outstanding scientific reports representing conscientious planning, hard work, and excellent execution of the final manuscript.

The original publishers are gratefully acknowledged for permitting reproduction of copyrighted material from their journals. These publishers are as follows:

Agriculture Research Service, U. S. Department of Agriculture, publishers of PLANT DISEASE REPORTER

Akademie-Verlag, publishers of INTERNATIONAL REVUE DER GESAMTEN HYDROBIOLOGIC AND HYDROGRAPHIC

The American Chemical Society, publishers of INDUSTRIAL AND ENGINEERING CHEMISTRY

American Fisheries Society, publishers of TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY

The American Public Health Association, Inc., publishers of AMERICAN JOURNAL OF PUBLIC HEALTH AND THE NATION'S HEALTH

American Society of Limnology and Oceanography, Inc., publishers of LIMNOLOGY AND OCEANOGRAPHY

American Water Works Association, Inc., publishers of JOURNAL AMERICAN WATER WORKS ASSOCIATION

Bureau of Commercial Fisheries, U.S. Department of The Interior (Formerly, Bureau of Fisheries, U.S. Department of Commerce), publishers of BULLETIN OF THE BUREAU OF FISHERIES

Bureau of Sport Fisheries and Wildlife, U.S. Department of the Interior, publishers of THE PROGRESSIVE FISH-CULTURIST

The Duke University Press, publishers of ECOLOGY

Gebrüder Borntraeger/Verlogsbuchhandlung, publishers of BERICHTE DER DEUTSCHEN BOTANISCHEN GESELLSCHAFT

Illinois Natural History Survey, publishers of ILLINOIS NATURAL HISTORY SURVEY BULLETIN

International Association of Milk, Food and Environmental Sanitarians, Inc., publishers of JOURNAL OF MILK AND FOOD TECHNOLOGY

New England Water Works Association, publishers of JOURNAL OF THE NEW ENGLAND WATER WORKS ASSOCIATION

The Ohio State University Engineering Experiment Station, publishers of PROCEEDINGS, FIRST OHIO WATER CLINIC, OHIO STATE UNIVERSITY ENGINEERING SERIES BULLETIN

Public Health Service, U.S. Department of Health, Education, and Welfare, publishers of PUBLIC HEALTH REPORTS

Public Works Journal Corp., publishers of PUBLIC WORKS

Purdue University, School of Civil Engineering, publishers of PURDUE UNIVERSITY ENGINEERING BULLETIN, PROCEEDINGS OF THE (annual) INDUSTRIAL WASTE CONFERENCE

The Water Pollution Control Federation, publishers of JOURNAL WATER POLLUTION CONTROL FEDERATION (Formerly cited, SEWAGE WORKS JOURNAL and SEWAGE AND INDUSTRIAL WASTES)

West Virginia Pulp and Paper, Chemical Division, publishers of TASTE AND ODOR CONTROL JOURNAL and TASTE AND ODOR CONTROL IN WATER PURIFICATION

The English translations of the two German articles were performed by the Joint Publications Research Service, U. S. Department of Commerce.

Special thanks are also extended to Mr. Richard W. Warner, Mrs. Martha Jean Wilkey, Mrs. Dorothy M. Williams, Mrs. Rosalynd J. Kendall, and Mrs. Jacquelyn P. Keup, who all contributed special skills and knowledge in obtaining materials and assembling the manuscript.

CONTENTS

PREFACE i

ACKNOWLEDGMENTS ii

I. GENERAL BACKGROUND OF BIOLOGICAL ASPECTS OF WATER POLLUTION 1

THE LAKE AS A MICROCOSM

S. A. Forbes 3

SEWAGE, ALGAE AND FISH

F. J. Brinley 10

BIOLOGICAL ASPECTS OF STREAM POLLUTION

A. F. Bartsch 13

SOME IMPORTANT BIOLOGICAL EFFECTS OF POLLUTION OFTEN DISREGARDED IN STREAM SURVEYS

C. M. Tarzwell and A. R. Gaufin 21

BIOLOGICAL INDICES OF WATER POLLUTION, WITH SPECIAL REFERENCE TO FISH POPULATIONS

P. Doudoroff and C. E. Warren 32

BIOACCUMULATION OF RADIOISOTOPES THROUGH AQUATIC FOOD CHAINS

J. J. Davis and R. F. Foster 41

II. RELATIONSHIP TO POLLUTION OF PLANKTON 47

ECOLOGY OF PLANT SAPROBIA

R. Kolkwitz and M. Marrson 47

EFFECTS OF SUNLIGHT AND GREEN ORGANISMS ON RE-AERATION OF STREAMS

W. Rudolfs and H. Heukelekian 52

THE PLANKTON OF THE SANGAMON RIVER IN THE SUMMER OF 1929

S. Eddy 57

AQUATIC LIFE IN WATERS POLLUTED BY ACID MINE WASTE

J. B. Lackey 75

A HEAVY MORTALITY OF FISHES RESULTING FROM THE DECOMPOSITION OF ALGAE IN THE YAHARA RIVER, WISCONSIN

K. M. Mackenthun, E. F. Herman, and A. F. Bartsch 77

SUGGESTED CLASSIFICATION OF ALGAE AND PROTOZOA IN SANITARY SCIENCE

C. M. Palmer and W. M. Ingram 79

III. RELATIONSHIP TO POLLUTION OF BOTTOM ORGANISMS 85

ECOLOGY OF ANIMAL SAPROBIA

R. Kolkwitz and M. Marsson 85

VALUE OF THE BOTTOM SAMPLER IN DEMONSTRATING THE EFFECTS OF POLLUTION ON FISH-FOOD ORGANISMS IN THE SHENANDOAH RIVER

C. Henderson 96

AQUATIC ORGANISMS AS AN AID IN SOLVING WASTE DISPOSAL PROBLEMS

R. Patrick 108

EFFECTS OF SILTATION, RESULTING FROM IMPROPER LOGGING ON THE BOTTOM FAUNA OF A SMALL TROUT STREAM IN THE SOUTHERN APPALACHIANS

L. B. Tebo, Jr. 114

III. RELATIONSHIP TO POLLUTION OF BOTTOM ORGANISMS (Continued)

STREAM LIFE AND THE POLLUTION ENVIRONMENT

A. F. Bartsch and W. M. Ingram 119

IV. RELATIONSHIP TO POLLUTION OF FISH 129

DETECTION AND MEASUREMENT OF STREAM POLLUTION

M. M. Ellis 129

THE EFFECTS OF SEWAGE POLLUTION ON THE FISH POPULATION OF A MIDWESTERN STREAM

M. Katz and A. R. Gaufin 186

THE EFFECTS OF ACID MINE POLLUTION ON THE FISH POPULATION OF GOOSE CREEK, CLAY COUNTY, KENTUCKY

W. R. Turner 192

V. RELATIONSHIP OF POLLUTION TO MAN 195

WATER QUALITY REQUIREMENTS FOR RECREATIONAL USES

A. H. Stevenson 195

WATER POLLUTION, ITS EFFECT ON THE PUBLIC HEALTH

J. D. Porterfield 198

POTENTIAL PLANT PATHOGENIC FUNGI IN SEWAGE AND POLLUTED WATER

W. B. Cooke 201

WATER-BORNE TYPHOID EPIDEMIC AT KEENE, NEW HAMPSHIRE

W. A. Healy and R. P. Crossman. 207

VI. BIOLOGY OF POTABLE WATER SUPPLIES. 215

TRANSFORMATIONS OF IRON BY BACTERIA IN WATER

R. L. Starkey. 215

CHEMICAL COMPOSITION OF ALGAE AND ITS RELATIONSHIP TO TASTE AND ODOR

G. A. Rohlich and W. B. Sarles 232

AQUATIC BIOLOGY AND THE WATER WORKS ENGINEER

J. B. Lackey 236

PRE-TREATMENT BASIN FOR ALGAE REMOVAL

A. J. Marx 239

INDUSTRIAL WASTES AS A SOURCE OF TASTES AND ODORS IN WATER SUPPLIES

A. M. Buswell 244

VII. BIOLOGY OF SEWAGE AND INDUSTRIAL WASTES TREATMENT 247

THE CHEMISTRY AND BIOLOGY OF MILK WASTE DISPOSAL

T. F. Wisniewski 247

PROTOZOA AND ACTIVATED SLUDGE

R. E. McKinney and A. Gram 252

BIOLOGICAL FACTORS IN TREATMENT OF RAW SEWAGE IN ARTIFICIAL PONDS

A. F. Bartsch and M. O. Allum. 262

TRICKLING FILTER ECOLOGY

W. B. Cooke 269

SELECTION AND ADAPTATION OF MICROORGANISMS IN WASTE TREATMENT

P. W. Kabler. 287

Chapter I

GENERAL BACKGROUND OF BIOLOGICAL ASPECTS OF WATER POLLUTION

Reproduced With Permission From:
ILLINOIS NATURAL HISTORY SURVEY BULLETIN
15(1925): 537-550

Preceding page blank

THE LAKE AS A MICROCOSM*

Stephen A. Forbes

A lake is to the naturalist a chapter out of the history of a primeval time, for the conditions of life there are primitive, the forms of life are, as a whole, relatively low and ancient, and the system of organic interactions by which they influence and control each other has remained substantially unchanged from a remote geological period.

The animals of such a body of water are, as a whole, remarkably isolated -- closely related among themselves in all their interests, but so far independant of the land about them that if every terrestrial animal were suddenly annihilated it would doubtless be long before the general multitude of the inhabitants of the lake would feel the effects of this event in any important way. It is an islet of older, lower life in the midst of the higher, more recent life of the surrounding region. It forms a little world within itself -- a microcosm within which all the elemental forces are at work and the play of life goes on in full, but on so small a scale as to bring it easily within the mental grasp.

Nowhere can one see more clearly illustrated what may be called the *sensibility* of such an organic complex, expressed by the fact that whatever affects any species belonging to it, must have its influence of some sort upon the whole assemblage. He will thus be made to see the impossibility of studying completely any form out of relation to the other forms; the necessity for taking a comprehensive survey of the whole as a condition to a satisfactory understanding of any part. If one wishes to become acquainted with the black bass, for example, he will learn but little if he limits himself to that species. He must evidently study also the species upon which it depends for its existence, and the various conditions upon which *these* depend. He must likewise study the species with which it comes in competition, and the entire system of conditions affecting their prosperity; and by the time he has studied all these sufficiently he will find that he has run through the whole complicated mechanism of the aquatic life of the locality, both animal and vegetable, of which his species forms but a single element.

It is under the influence of these general ideas that I propose to examine briefly to-night the lacustrine life of Illinois, drawing my data from collections and observations made during recent years by myself and my assistants of the State Laboratory of Natural History.

The lakes of Illinois are of two kinds, fluviatile and water-shed. The fluviatile lakes, which are much the more numerous and important, are appendages of the river systems of the state, being situated in the river bottoms and connected with the adjacent streams by periodical overflows. Their fauna is therefore substantially that of the rivers themselves, and the two should, of course, be studied together.

They are probably in all cases either parts of former river channels, which have been cut off and abandoned by the current as the river changed its course, or else are tracts of the high-water beds of streams over which, for one reason or another, the periodical deposit of sediment has gone on less rapidly than over the surrounding area, and which have thus come to form depressions in the surface which retain the waters of overflow longer than the higher lands adjacent. Most of the numerous "horseshoe lakes" belong to the first of these varieties, and the "bluff-lakes," situated along the borders of the bottoms, are many of them examples of the second.

These fluviatile lakes are most important breeding grounds and reservoirs of life, especially as they are protected from the filth and poison of towns and manufactories by which the running waters of the state are yearly more deeply defiled.

The amount and variety of animal life contained in them as well as in the streams related to them is extremely variable, depending chiefly on the frequency, extent, and duration of the spring and summer overflows. This is, in fact, the characteristic and peculiar feature of life in these waters. There is perhaps no better illustration of the methods by which the flexible system of organic life adapts itself, without injury, to widely and rapidly fluctuating conditions. Whenever the waters of the river remain for a long time far beyond their banks, the breeding grounds of fishes and other animals are immensely extended, and their food supplies increased to a corresponding degree. The slow or stagnant backwaters of such an overflow afford the best situations possible for the development of myriads of Entomostraca, which furnish, in turn, abundant food for young fishes of all descriptions. There thus results an outpouring of life -- an extraordinary multiplication of nearly every species, most prompt and rapid, generally speaking, in such as have the highest reproductive rate, that is to say, in those which produce the largest average number of eggs and young for each adult.

---

*This paper, originally read February 25, 1887, to the Peoria Scientific Association (now extinct), and published in their Bulletin, was reprinted many years ago by the Illinois State Laboratory of Natural History in an edition which has long been out of print. A single copy remaining in the library of the Natural History Survey is used every year by classes in the University of Illinois, and a professor of zoology in a Canadian university borrows a copy regularly from a Peoria library for use in his own classes. In view of this long-continued demand and in the hope that the paper may still be found useful elsewhere, it is again reprinted, with trivial emendations, and with no attempt to supply its deficiencies or to bring it down to date.

The first to feel this tremendous impulse are the protophytes and Protozoa, upon which most of the Entomostraca and certain minute insect larvae depend for food. This sudden development of their food resources causes, of course, a corresponding increase in the numbers of the latter classes, and, through them, of all sorts of fishes. The first fishes to feel the force of this tidal wave of life are the rapidly-breeding, non-predaceous kinds; and the last, the game fishes, which derive from the others their principal food supplies. Evidently each of these classes must act as a check upon the one preceding it. The development of animalcules is arrested and soon sent back below its highest point by the consequent development of Entomostraca; the latter, again, are met, checked, and reduced in number by the innumerable shoals of fishes with which the water speedily swarms. In this way a general adjustment of numbers to the new conditions would finally be reached spontaneously; but long before any such settled balance can be established, often of course before the full effect of this upward influence has been exhibited, a new cause of disturbance intervenes in the disappearance of the overflow. As the waters retire, the lakes are again defined; the teeming life which they contain is restricted within daily narrower bounds, and a fearful slaughter follows; the lower and more defenseless animals are penned up more and more closely with their predaceous enemies, and these thrive for a time to an extraordinary degree. To trace the further consequences of this oscillation would take me too far. Enough has been said to illustrate the general idea that the life of waters subject to periodical expansions of considerable duration, is peculiarly unstable and fluctuating; that each species swings, pendulum-like but irregularly, between a highest and a lowest point, and that this fluctuation affects the different classes successively, in the order of their dependence upon each other for food.

Where a water-shed is a nearly level plateau with slight irregularities of the surface many of these will probably be imperfectly drained, and the accumulating waters will form either marshes or lakes according to the depth of the depressions. Highland marshes of this character are seen in Ford, Livingston, and adjacent counties,* between the headwaters of the Illinois and Wabash systems and an area of water-shed lakes occurs in Lake and McHenry counties, in northern Illinois.

The latter region is everywhere broken by low, irregular ridges of glacial drift, with no rock but boulders anywhere in sight. The intervening hollows are of every variety, from mere sink-holes, either dry or occupied by ponds, to expanses of several square miles, forming marshes or lakes.

This is, in fact, the southern end of a broad lake belt which borders Lakes Michigan and Superior on the west and south, extending through eastern and northern Wisconsin and northwestern Minnesota, and occupying the plateau which separates the headwaters of the St. Lawrence from those of the Mississippi. These lakes are of glacial origin, some filling beds excavated in the solid rock, and others collecting the surface waters in hollows of the drift. The latter class, to which all the Illinois lakes belong, may lie either parallel to the line of glacial action, occupying valleys between adjacent lateral moraines, or transverse to that line and bounded by terminal moraines. Those of our own state all drain at present into the Illinois through the Des Plaines and Fox; but as the terraces around their borders indicate a former water-level considerably higher than the present one it is likely that some of them once emptied eastward into Lake Michigan. Several of these lakes are clear and beautiful sheets of water, with sandy or gravelly beaches, and shores bold and broken enough to relieve them from monotony. Sportsmen long ago discovered their advantages and club-houses and places of summer resort are numerous on the borders of the most attractive and easily accessible. They offer also an unusually rich field to the naturalist, and their zoology and botany should be better known.

The conditions of aquatic life are here in marked contrast to those afforded by the fluviatile lakes already mentioned. Connected with each other or with adjacent streams only by slender rivulets, varying but little in level with the change of the season and scarcely at all from year to year, they are characterized by an isolation, independence, and uniformity which can be found nowhere else within out limits.

Among these Illinois lakes I did considerable work during October of two successive years, using the sounding line, deep-sea thermometer, towing net, dredge, and trawl in six lakes of northern Illinois, and in Geneva Lake, Wisconsin, just across the line. Upon one of these Illinois lakes I spent a week in October, and an assistant, Prof. H. Garman, now of the University, spent two more, making as thorough a physical and zoological survey of this lake as was possible at that season of the year.

I now propose to give you in this paper a brief general account of the physical characters and the fauna of these lakes, and of the relations of the one to the other; to compare, in a general way, the animal assemblages which they contain with those of Lake Michigan -- where also I did some weeks of active aquatic work in 1881 -- and with those of the fluviatile lakes of central Illinois; to make some similar comparisons with the lakes of Europe; and, finally, to reach the subject which has given the title to this paper -- to study the system of natural interactions by which this mere collocation of plants and animals has been organized as a stable and prosperous community.

First let us endeavor to form the mental picture. To make this more graphic and true to the facts, I will describe to you some typical lakes among those in which we worked; and will then do what I can to furnish you the materials for a picture of the life that swims and creeps and crawls and burrows and climbs through the water, in and on the bottom, and among the feathery water-plants with which large areas of these lakes are filled.

---

* All now drained and brought under cultivation.

Fox Lake, in the western border of Lake county, lies in the form of a broad irregular crescent, truncate at the ends, and with the concavity of the crescent to the northwest. The northern end is broadest and communicates with Petite Lake. Two points projecting inward from the southern shore form three broad bays. The western end opens into Nippisink Lake, Crab Island separating the two. Fox River enters the lake from the north, just eastward of this island, and flows directly through the Nippisink. The length of a curved line extending through the central part of this lake, from end to end, is very nearly three miles, and the width of the widest part is about a mile and a quarter. The shores are bold, broken, and wooded, except to the north, where they are marshy and flat. All the northern and eastern part of the lake was visibly shallow -- covered with weeds and feeding water-fowl, and I made no soundings there. The water there was probably nowhere more than two fathoms in depth, and over most of that area was doubtless under one and a half. In the western part, five lines of soundings were run, four of them radiating from Lippincott's Point, and the fifth crossing three of these nearly at right angles. The deepest water was found in the middle of the mouth of the western bay, where a small area of five fathoms occurs. On the line running northeast from the Point, not more than one and three fourths fathoms is found. The bottom at a short distance from the shores was everywhere a soft, deep mud. Four hauls of the dredge were made in the western bay, and the surface net was dragged about a mile.

Long Lake differs from this especially in its isolation, and in its smaller size. It is about a mile and a half in length by a mile in breadth. Its banks are all bold except at the western end, where a marshy valley traversed by a small creek connects it with Fox Lake, at a distance of about two miles. The deepest sounding made was six and a half fathoms, while the average depth of the deepest part of the bed was about five fathoms.

Cedar Lake, upon which we spent a fortnight, is a pretty sheet of water, the head of a chain of six lakes which open finally into the Fox. It is about a mile in greatest diameter in each direction, with a small but charming island bank near the center, covered with bushes and vines -- a favorite home of birds and wild flowers. The shores vary from rolling to bluffy except for a narrow strip of marsh through which the outlet passes, and the bottoms and margins are gravel, sand, and mud in different parts of its area. Much of the lake is shallow and full of water plants; but the southern part reaches a depth of fifty feet a short distance from the eastern bluff.

Deep Lake, the second of this chain, is of similar character, with a greatest depth of fifty-seven feet -- the deepest sounding we made in these smaller lakes of Illinois. In these two lakes several temperatures were taken with a differential thermometer. In Deep Lake, for example, at fifty-seven feet I found the bottom temperature 53-1/2° -- about that of ordinary well-water -- when the air was 63°; and in Cedar Lake, at forty-eight feet, the bottom was 58° when the air was 61°.

Geneva Lake, Wisconsin, is a clear and beautiful body of water about eight miles long by one and a quarter in greatest width. The banks are all high, rolling, and wooded, except at the eastern end, where its outlet rises. Its deepest water is found in its western third, where it reaches a depth of twenty-three fathoms. I made here, early in November, twelve hauls of the dredge and three of the trawl, aggregating about three miles in length, so distributed in distance and depth as to give a good idea of the invertebrate life of the lake at that season.

And now if you will kindly let this suffice for the background or setting of the picture of lacustrine life which I have undertaken to give you, I will next endeavor -- not to paint in the picture; for that I have not the artistic skill. I will confine myself to the humble and safer task of supplying you the pigments, leaving it to your own constructive imaginations to put them on the canvas.

When one sees acres of the shallower water black with water-fowl, and so clogged with weeds that a boat can scarcely be pushed through the mass; when, lifting a handful of the latter, he finds them covered with shells and alive with small crustaceans; and then, dragging a towing net for a few minutes, finds it lined with myriads of diatoms and other microscopic algae, and with multitudes of Entomostraca, he is likely to infer that these waters are everywhere swarming with life, from top to bottom and from shore to shore. If, however, he will haul a dredge for an hour or so in the deepest water he can find, he will invariably discover an area singularly barren of both plant and animal life, yielding scarcely anything but a small bivalve mollusk, a few low worms, and red larvae of gnats. These inhabit a black, deep, and almost impalpable mud or ooze, too soft and unstable to afford foothold to plants even if the lake is shallow enough to admit a sufficient quantity of light to its bottom to support vegetation. It is doubtless to this character of the bottom that the barrenness of the interior parts of these lakes is due; and this again is caused by the selective influence of gravity upon the mud and detritus washed down by rains. The heaviest and coarsest of this material necessarily settles nearest the margin, and only the finest silt reaches the remotest parts of the lakes, which, filling most slowly, remain, of course, the deepest. This ooze consists very largely, also, of a fine organic debris. The superficial part of it contains scarcely any sand, but has a greasy feel and rubs away, almost to nothing, between the fingers. The largest lakes are not therefore, as a rule, by any means the most prolific of life, but this shades inward rapidly from the shore, and becomes at no great distance almost as simple and scanty as that of a desert.

Among the weeds and lily-pads upon the shallows and around the margin -- the Potamogeton, Myriophyllum, Ceratophyllum, Anacharis, and Chara, and the common Nelumbium, -- among these the fishes chiefly swim or lurk, by far the commonest being the barbaric bream[1] or "pumpkin-seed" of northern Illinois, splendid with its green and scarlet and purple and orange. Little less abundant is the common perch (Perca lutea) in the larger lakes -- in the largest outnumbering the bream itself. The whole sunfish family, to which the latter belongs, is in fact the dominant group in these lakes. Of the one hundred and thirty-two fishes of Illinois only thirty-seven are found in these waters -- about twenty-eight per cent -- while eight out of our seventeen sunfishes (Centrarchinae) have been taken there. Next, perhaps, one searching the pebbly beaches or scanning the weedy tracts will be struck by the small number of minnows or cyprinoids which catch the eye or come out in the net. Of our thirty-three Illinois cyprinoids, only six occur there -- about eighteen per cent -- and only three of these are common. These are in part replaced by shoals of the beautiful little silversides (Labidesthes sicculus), a spiny-finned fish, bright, slender, active, and voracious -- as well supplied with teeth as a perch, and far better equipped for self-defense than the soft-bodied and toothless cyprinoids. Next we note that of our twelve catfishes (Siluridae) only two have been taken in these lakes -- one the common bullhead (Ictalurus nebulosus), which occurs everywhere, and the other an insignificant stone cat, not as long as one's thumb. The suckers, also, are much less abundant in this region than farther south, the buffalo fishes[1] not appearing at all in our collections. Their family is represented by worthless carp[2] by two red-horse[3], by the chub sucker[4] and the common sucker (Catostomus teres), and by one other species. Even the hickory shad[5] -- an ichthyological weed in the Illinois -- we have not found in these lakes at all. The sheepshead[6], so common here, is also conspicuous there by its absence. The yellow bass[7], not rare in this river, we should not expect in these lakes because it is, rather, a southern species; but why the white bass[8], abundant here, in Lake Michigan, and in the Wisconsin lakes, should be wholly absent from the lakes of the Illinois plateau, I am unable to imagine. If it occurs there at all, it must be rare, as I could neither find nor hear of it.

---

[1] Lepomis gibbosus.

A characteristic, abundant, and attractive little fish is the log perch (Percina caprodes) -- the largest of the darters, slender, active, barred like a zebra, spending much of its time in chase of Entomostraca among the water plants, or prying curiously about among the stones for minute insect larvae. Six darters in all (Ethcostomatinae), out of the eighteen from the state, are on our list from these lakes. The two black bass[9] are the most popular game fishes -- the large-mouthed species being much the most abundant. The pickerels[10], gar[11], and dogfish[12] are there about as here; but the shovel-fish[13] does not occur.

Of the peculiar fish fauna of Lake Michigan -- the burbot[14], white fish[15], trout[16], lake herring or cisco[17], etc., not one species occurs in these smaller lakes, and all attempts to transfer any of them have failed completely. The cisco is a notable fish of Geneva Lake, Wisconsin, but does not reach Illinois except in Lake Michigan. It is useless to attempt to introduce it, because the deeper areas of the interior lakes are too limited to give it sufficient range of cool water in midsummer.

In short, the fishes of these lakes are substantially those of their region -- excluding the Lake Michigan series (for which the lakes are too small and warm) and those peculiar to creeks and rivers. Possibly the relative scarcity of catfishes (Siluridae) is due to the comparative clearness and cleanness of these waters. I see no good reason why minnows should be so few, unless it be the abundance of pike and Chicago sportsmen.

Concerning the molluscan fauna, I will only say that it is poor in bivalves -- as far as our observations go -- and rich in univalves. Our collections have been but partly determined, but they give us three species of Valvata, seven of Planorbis, four Amnicolas, a Melantho, two Physas, six Limnaeas, and an Ancylus among the Gastropoda, and two Unios, an Anodonta, a Sphaerium, and a Pisidium among the Lamellibranchiates. Pisiduim variabile is by far the most abundant mollusk in the oozy bottom in the deeper parts of the lakes; and crawling over the weeds are multitudes of small Amnicolas and Valvatas.

The entomology of these lakes I can merely touch upon, mentioning only the most important and abundant insect larvae. Hiding under stones and driftwood, well aware, no doubt, what enticing morsels they are to a great variety of fishes, we find a number of species of ephemerid larvae whose specific determination we have not yet attempted. Among the weeds are the usual larvae of dragon-flies -- Agrionina and Libellulina, familiar to every one; swimming in open water the predaceous larvae of Corethra; wriggling through the water or buried in the mud the larvae of Chironomus -- the shallow water species white, and those from the the deeper ooze of the central parts of the lakes blood-red and larger. Among Chara on the sandy bottom are a great number and variety of interesting case-worms -- larvae of Phryganeidae -- most of them inhabiting tubes of a slender conical form made of a viscid secretion exuded from the mouth and strengthened and thickened by grains of sand, fine or coarse. One of these cases, nearly naked, but usually thinly covered with diatoms, is especially worthy of note, as it has been reported nowhere in this country except in our collections, and was indeed recently described from Brazil as new. Its generic name is Lagenopsyche, but its species undertermined. These larvae are also eaten by fishes.

Among the worms we have of course a number of species of leeches and of planarians, -- in the mud minute Anguillulidae, like vinegar eels, and a slender Lumbriculus which makes a tubular mud burrow for itself in the deepest water, and also the curious Nais probiscidea, notable for its capacity of multiplication by transverse division.

The crustacean fauna of these lakes is more varied than any other group. About forty species were noted

[1] Ictiobus bubalus. [2] Ictiobus cyprinus. [3] Moxostoma aureolum and M. macrolepidotum. [4] Erimyzon sucetta. [5] Dorosoma cepedianum. [6] Haploidonotus. [7] Roccus interruptus. [8] Roccus chrysops. [9] Micropterus. [10] Esox. [11] Lepidosteus. [12] Amia. [13] Polyodon. [14] Lota. [15] Coregonus clupeiformis. [16] Salvelinus namaycush. [17] Coregonus artedi.

in all. Crawfishes were not especially abundant, and most belonged to a single species, Cambarus virilis. Two amphipods occurred frequently in our collections; one, less common here but very abundant farther south -- Crangonyx gracilis -- and one, Allorchestes dentata, probably the commonest animal in these waters, crawling and swimming everywhere in myriads among the submerged water-plants. An occasional Gammarus fasciatus was also taken in the dredge. A few isopod Crustacea occur, belonging to Mancasellus tenax -- a species not previously found in the state.

I have reserved for the last the Entomostraca -- minute crustaceans of a surprising number and variety, and of a beauty often truly exquisite. They belong wholly, in our waters, to the three orders, Copedoda, Ostracoda, and Cladocera -- the first two predaceous upon still smaller organisms and upon each other, and the last chiefly vegetarian. Twenty-one species of Cladocera have been recognized in our collections, representing sixteen genera. It is an interesting fact that twelve of these species are found also in the fresh waters of Europe. Five cyprids have been detected, two of them common to Europe, and also an abundant Diaptomus, a variety of a European species. Several Cyclops species were collected which have not yet been determined.

These Entomostraca swarm in microscopic myriads among the weeds along the shore, some swimming freely, and others creeping in the mud or climbing over the leaves of plants. Some prefer the open water, in which they throng locally like shoals of fishes, coming to the surface preferably by night, or on dark days, and sinking to the bottom usually by day to avoid the sunshine. These pelagic forms, as they are called, are often exquisitely transparent, and hence almost invisible in their native element -- a charming device of Nature to protect them against their enemies in the open lake, where there is no chance of shelter or escape. Then with an ingenuity in which one may almost detect the flavor of sarcastic humor, Nature has turned upon these favored children and endowed their most deadly enemies with a like transparency, so that wherever the towing net brings to light a host of these crystalline Cladocera, there it discovers also swimming, invisible, among them, a lovely pair of robbers and beasts of prey -- the delicate Leptodora and the Corethra larva.

These slight, transparent, pelagic forms are much more numerous in Lake Michigan than in any of the smaller lakes, and peculiar forms occur there commonly which are rare in the larger lakes of Illinois and entirely wanting in the smallest. The transparent species are also much more abundant in the isolated smaller lakes than in those more directly connected with the rivers.

The vertical range of the animals of Geneva Lake showed clearly that the barrenness of the interiors of these small bodies of water was not due to the greater depth alone. While there were a few species of crustaceans and case-worms which occurred there abundantly near shore but rarely or not at all at depths greater than four fathoms, and may hence be called littoral species, there was, on the whole, little diminution either in quantity or variety of animal life until about fifteen fathoms had been reached. Dredging at four or five fathoms were nearly or quite as fruitful as any made. On the other hand, the barrenness of the bottom at twenty to twenty-three fathoms was very remarkable The total product of four hauls of the dredge and one of the trawl at that depth, aggregating fully a mile and a half of continuous dragging, would easily go into a two-dram vial, and represents only nine animal species -- not counting dead shells, and fragments which had probably floated in from shallower waters. The greater part of this little collection was composed of specimens of Lumbriculus and larvae of Chironomus. There were a few Corethra larvae, a single Gammarus, three small leeches, and some sixteen mollusks, all but four of which belonged to Pisidium. The others were two Sphaeriums, a Valvata carinata, and a V. sincera. None of the species taken here are peculiar, but all were of the kinds found in the smaller lakes, and all occurred also in shallower water. It is evident that these interior regions of the lakes must be as destitute of fishes as they are of plants and lower animals.

While none of the deep-water animals of the Great Lakes were found in Geneva Lake, other evidences of zoological affinity were detected. The towing net yielded almost precisely the assemblage of species of Entomostraca found in Lake Michigan, including many specimens of Limnocalanus macrurus Sars; and peculiar long, smooth leeches, common in Lake Michigan but not occurring in the small Illinois lakes, were also found in Geneva. Many Valvata tri-carinata lacked the middle carina, as in Long Lake and other isolated lakes of this region.

Comparing the Daphnias of Lake Michigan with those of Geneva Lake, Wis. (nine miles long and twenty-three fathoms in depth), those of Long Lake, Ill. (one and a half miles long and six fathoms deep), and those of other, still smaller, lakes of that region, and the swamps and smaller ponds as well, we shall be struck by the inferior development of the Entomostraca of the larger bodies of water in numbers, in size and robustness, and in reproductive power. Their smaller numbers and size are doubtless due to the relative scarcity of food. The system of aquatic animal life rests essentially upon the vegetable world, although perhaps less strictly than does the terrestrial system, and in a large and deep lake vegetation is much less abundant than in a narrower and shallower one, not only relatively to the amount of water but also to the area of the bottom. From this deficiency of plant life results a deficiency of food for Entomostraca, whether of algae, of Protozoa, or of higher forms, and hence, of course, a smaller number of the Entomostraca themselves, and these with more slender bodies, suitable for more rapid locomotion and wider range.

The difference of reproductive energy, as shown by the much smaller egg-masses borne by the species of the larger lakes, depends upon the vastly greater destruction to which the paludal Crustacea are subjected. Many of the latter occupy waters liable to be exhausted by drought, with a consequent enormous waste of entomostracan life. The opportunity for reproduction is here greatly limited -- in some situations to early spring alone -- and the chances for destruction of the summer eggs in the dry and often dusty soil are so numerous that only the most prolific species can maintain themselves.

Further, the marshes and shallower lakes are the favorite breeding grounds of fishes, which migrate to them in spawning time if possible, and it is from the Entomostraca found here that most young fishes get their earliest food supplies -- a danger from which the deep-water species are measurably free. Not only is a high reproductive rate rendered unnecessary among the latter by their freedom from many dangers to which the shallow-water species are exposed, but in view of the relatively small amount of food available for them, a high rate of multiplication would be a positive injury, and could result only in wholesale starvation.

All these lakes of Illinois and Wisconsin, together with the much larger Lake Mendota at Madison (in which also I have done much work with dredge, trawl, and seine), differ in one notable particular both from Lake Michigan and from the larger lakes of Europe. In the latter the bottoms in the deeper parts yield a peculiar assemblage of animal forms which range but rarely into the littoral region, while in our inland lakes no such deep water fauna occurs, which the exception of the cisco and the large red Chironomus larva. At Grand Traverse Bay, in Lake Michigan, I found at a depth of one hundred fathoms a very odd fish of the sculpin family (Triglopsis thompsoni Gir.) which, until I collected it, had been known only from the stomachs of fishes; and there also was an abundant crustacean, Mysis -- the "opossum shrimp", as it is sometimes called -- the principal food of these deep lake sculpins. Two remarkable amphipod crustaceans also belong in a peculiar way to this deep water. In the European lakes the same Mysis occurs in the deepest part, with several other forms not represented in our collections, two of these being blind crustaceans related to those which in this country occur in caves and wells.

Comparing the other features of our lake fauna with that of Europe, we find a surprising number of Entomostraca identical; but this is a general phenomenon, as many of the more abundant Cladocera and Copepoda of our small wayside pools are either European species, or differ from them so slightly that it is doubtful if they ought to be called distinct.

It would be quite impossible, within reasonable limits, to go into details respecting the organic relations of the animals of these waters, and I will content myself with two or three illustrations. As one example of the varied and far-reaching relations into which the animals of a lake are brought in the general struggle for life, I take the common black bass. In the dietary of this fish I find, at different ages of the individual, fishes of great variety, representing all the important orders of that class; insects in considerable number, especially the various water-bugs and larvae of day-flies; fresh-water shrimps; and a great multitude of Entomostraca of many species and genera. The fish is therefore directly dependent upon all these classes for its existence. Next, looking to the food of the species which the bass has eaten, and upon which it is therefore indirectly dependent, I find that one kind of the fishes taken feeds upon mud, algae, and Entomostraca, and another upon nearly every animal substance in the water, including mollusks and decomposing organic matter. The insects taken by the bass, themselves take other insects and small Crustacea. The crawfishes are nearly omnivorous, and of the other crustaceans some eat Entomostraca and some algae and Protozoa. At only the second step, therefore, we find our bass brought into dependence upon nearly every class of animals in the water.

And now, if we search for its competitors we shall find these also extremely numerous. In the first place, I have found that all our young fishes except the Catostomidae feed at first almost wholly on Entomostraca, so that the little bass finds himself at the very beginning of his life engaged in a scramble for food with all the other little fishes in the lake. In fact, not only young fishes but a multitude of other animals as well, especially insects and the larger Crustacea, feed upon these Entomostraca, so that the competitors of the bass are not confined to members of its own class. Even mollusks, while they do not directly compete with it do so indirectly, for they appropriate myriads of the microscopic forms upon which the Entomostraca largely depend for food. But the enemies of the bass do not all attack it by appropriating its food supplies, for many devour the little fish itself. A great variety of predaceous fishes, turtles, water-snakes, wading and diving birds, and even bugs of gigantic dimensions destroy it on the slightest opportunity. It is in fact hardly too much to say that fishes which reach maturity are relatively as rare as centenarians among human kind.

As an illustration of the remote and unsuspected rivalries which reveal themselves on a careful study of such a situation, we may take the relations of fishes to the bladderwort[1] -- a flowering plant which fills many acres of the water in the shallow lakes of northern Illinois. Upon the leaves of this species are found little bladders -- several hundred to each plant -- which when closely examined are seen to be tiny traps for the capture of Entomostraca and other minute animals. The plant usually has no roots, but lives entirely upon the animal food obtained through these little bladders. Ten of these sacs which I took at random from a mature plant contained no less than ninety-three animals (more than nine to a bladder), belonging to twenty-eight different species. Seventy-six of these were Entomostraca, and eight others were minute insect larvae. When we estimate the myriads of small insects and Crustacea which these plants must appropriate during a year to their own support, and consider the fact that these are of the kinds most useful as food for young fishes of nearly all descriptions, we must conclude that the bladderworts compete with fishes for food, and tend to keep down their number by diminishing the food resources of the young. The plants even have a certain advantage in this competition, since they are not strictly dependent on Entomostraca, as the fishes are, but sometimes take root, developing then but very few leaves and bladders. This probably happens under conditions unfavorable to their support by the other method. These simple instances will suffice to illustrate the intimate way in which the living forms of a lake are united.

---

[1] Utricularia.

Perhaps no phenomenon of life in such a situation is more remarkable than the steady balance of organic nature, which holds each species within the limits of a uniform average number, year after year, although each one is always doing its best to break across boundaries on every side. The reproductive rate is usually enormous and the struggle for existence is correspondingly severe. Every animal within these bounds has its enemies, and Nature seems to have taxed her skill and ingenuity to the utmost to furnish these enemies with contrivances for the destruction of their prey in myriads. For every defensive device with which she has armed an animal, she has invented a still more effective apparatus of destruction and bestowed it upon some foe, thus striving with unending pertinacity to outwit herself; and yet life does not perish in the lake, nor even oscillate to any considerable degree, but on the contrary the little community secluded here is as prosperous as if its state were one of profound and perpetual peace. Although every species has to fight its way inch by inch from the egg to maturity, yet no species is exterminated, but each is maintained at a regular average number which we shall find good reason to believe is the greatest for which there is, year after year, a sufficient supply of food.

I will bring this paper to a close, already too long postponed, by endeavoring to show how this beneficent order is maintained in the midst of a conflict seemingly so lawless.

It is a self-evident proposition that a species can not maintain itself continuously, year after year, unless its birth-rate at least equals its death-rate. If it is preyed upon by another species, it must produce regularly an excess of individuals for destruction, or else it must certainly dwindle and disappear. On the other hand, the dependent species evidently must not appropriate, on an average, any more than the surplus and excess of individuals upon which it preys, for if it does so it will continuously diminish its own food supply, and thus indirectly but surely exterminate itself. The interests of both parties will therefore be best served by an adjustment of their respective rates of multiplication such that the species devoured shall furnish an excess of numbers to supply the wants of the devourer, and that the latter shall confine its appropriations to the excess thus furnished. We thus see that there is really a close community of interest between these two seemingly deadly foes.

And next we note that this common interest is promoted by the process of natural selection; for it is the great office of this process to eliminate the unfit. If two species standing to each other in the relation of hunter and prey are or become badly adjusted in respect to their rates of increase, so that the one preyed upon is kept very far below the normal number which might find food, even if they do not presently obliterate each other the pair are placed at a disadvantage in the battle for life, and must suffer accordingly. Just as certainly as the thrifty business man who lives within his income will finally dispossess his shiftless competitor who can never pay his debts, the well-adjusted aquatic animal will in time crowd out its poorly-adjusted competitors for food and for the various goods of life. Consequently we may believe that in the long run and as a general rule those species which have survived, are those which have reached a fairly close adjustment in this particular.[1]

Two ideas are thus seen to be sufficient to explain the order evolved from this seeming chaos; the first that of a general community of interests among all the classes of organic beings here assembled, and the second that of the beneficent power of natural selection which compels such adjustments of the rates of destruction and of multiplication of the various species as shall best promote this common interest.

Have these facts and ideas, derived from a study of our aquatic microcosm, any general application on a higher plane? We have here an example of the triumphant beneficence of the laws of life applied to conditions seemingly the most unfavorable possible for any mutually helpful adjustment. In this lake, where competitions are fierce and continuous beyond any parallel in the worst periods of human history; where they take hold, not on goods of life merely, but always upon life itself; where mercy and charity and sympathy and magnanimity and all the virtues are utterly unknown; where robbery and murder and the deadly tyranny of strength over weakness are the unvarying rule; where what we call wrong-doing is always triumphant, and what we call goodness would be immediately fatal to its possessor, -- even here, out of these hard conditions, an order has been evolved which is the best conceivable without a total change in the conditions themselves; an equilibrium has been reached and is steadily maintained that actually accomplishes for all the parties involved the greatest good which the circumstances will at all permit. In a system where life is the universal good, but the destruction of life the well-nigh universal occupation, an order has spontaneously arisen which constantly tends to maintain life at the highest limit -- a limit far higher, in fact, with respect to both quality and quantity, than would be possible in the absence of this destructive conflict. Is there not, in this reflection, solid ground for a belief in the final beneficence of the laws of organic nature? If the system of life is such that a harmonious balance of conflicting interests has been reached where every element is either hostile or indifferent to every other, may we not trust much to the outcome where, as in human affairs, the spontaneous adjustments of nature are aided by intelligent effort, by sympathy, and by self-sacrifice?

---

[1] For a fuller statement of this argument, see Bul. Ill. State Lab. Nat. Hist. Vol. I, No. 3, pages 5 to 10.

Reproduced With Permission From:
SEWAGE WORKS JOURNAL
15(1943): 78-83

SEWAGE, ALGAE AND FISH*

Floyd J. Brinley

U. S. Public Health Service, Cincinnati, Ohio

Much has been written concerning the effects of stream pollutants on fish life. It is the general belief that fish cannot live in a stream polluted by domestic sewage or industrial wastes. The conservationists would like to have all waste materials prevented from entering out streams, thereby returning them, at least partially, to their virgin state. This, of course, would be ideal, but our population has increased to more than one hundred million people, conditions have changed and the clock cannot be turned backwards. Few people, however, seem to realize that domestic sewage, after proper treatment, increases the stream's biological productivity as represented by the plankton and fish population. It is the purpose of this paper to show that treated domestic sewage acts as a fertilizer for a stream in much the same manner as barnyard manure does for field plants.

While making a biological survey of the Ohio River Watershed the author was able to collect a large amount of data on the relation of domestic sewage to aquatic life. The present paper is based upon the data presented in detail in a supplement of a forthcoming report of the Ohio River Pollution Survey (1).

RELATION OF SEWAGE TO ALGAE

The entrance of untreated domestic sewage produces a well defined series of physical, chemical and biological changes in a flowing stream (2). In heavily polluted streams, the region immediately below the source of pollution is characterized by a high bacterial population. The water frequently has a cloudy appearance, high biochemical oxygen demand and a strong disagreeable odor, all indicating general depletion of dissolved oxygen. Masses of gaseous sludge, rising from the bottom of the more sluggish streams, are often noticed floating near the surface of the water. The plankton population in this region is composed largely of bacteria-eating ciliated protozoa, such as Paramecium and Colpidium. Large numbers of stalked ciliates (Vorticella and Carchesium) are frequently found attached to bottom objects. Colorless flagellates may be abundant, with an occasional chlorophyll-bearing species. The total volume of plankton is usually less than 2000 parts per million, but may reach several times that figure if conditions are optimum for the development of large numbers of protozoa. Long streamers of sewage fungus are frequently attached to submerged objects. The fishes that normally penetrate this region are carp and buffalo and they are found near the sewer outlet, feeding upon the raw sewage, where the bacterial action has not yet depleted the dissolved oxygen. These fish survive the prevailing low oxygen concentration by coming to the surface to "gulp" air.

Farther down stream, after sufficient time has elapsed for the masses of bacteria to decompose the sewage, the water tends to become clear and the dissolved oxygen level is sufficiently high to support forage and rough fish. The plankton population is slightly higher than upstream but is still composed largely of ciliated protozoa and colorless flagellates. Chlorophyll-bearing species are beginning to make their appearance in noticeable numbers. Blue-green and filamentous green algae are commonly found along the margins and bottom of the stream. Accumulated oxygen may bring large masses of the bottom algae to the surface of the water and give to the stream an unsightly appearance. (These floating islands of algae should not be confused with the gaseous sludge masses previously mentioned.) The combined photosynthetic action of all the green plants is an important factor in raising the oxygen level, especially on bright sunny days.

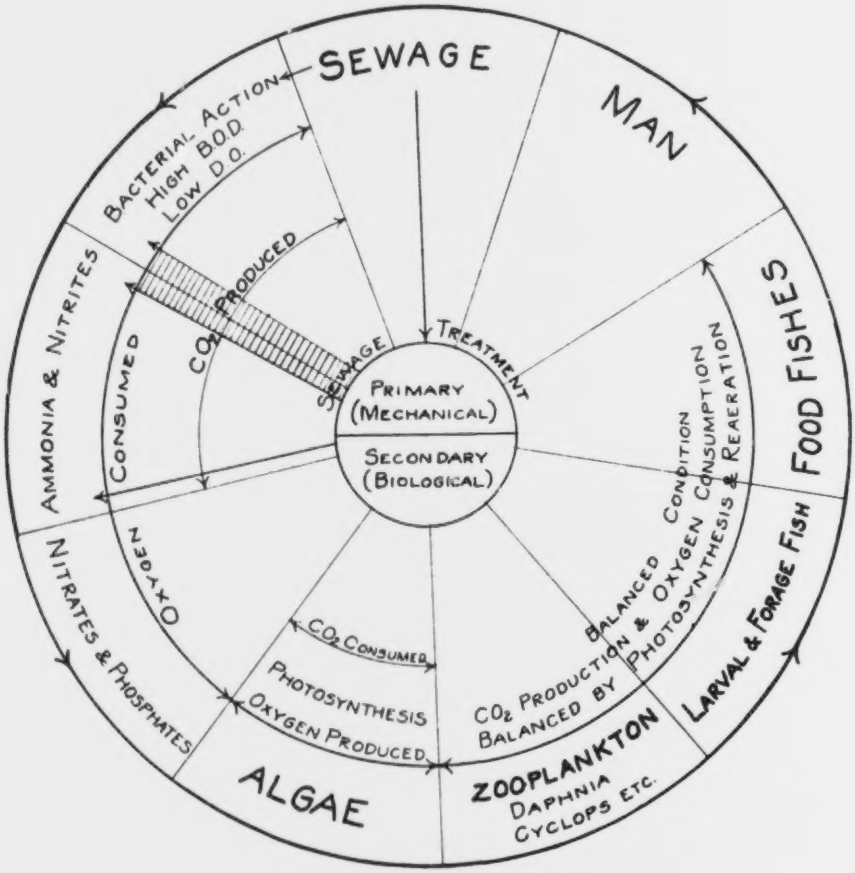
The adjacent region farther downstream clearly shows the beneficial effect of the decomposed sewage which entered upstream. The bacterial action in the upper reaches of the stream has oxidized the complex organic compounds present in the sewage to nitrates and phosphates. The availability of these end products as plant foods results in the development of large numbers of chlorophyll-bearing algae, which furnish food for the zooplankton and this food supply results in an increase in the population of mixed fishes (Fig. 1). The photosynthetic action of the green algae in this region increases the dissolved oxygen, often to supersaturation during the day, which, however, decreases at night but seldom to the asphyxial level for fishes.

Still farther downstream the plankton population drops sharply, probably owing largely to the utilization of the available food materials by the heavy growth of plankton in the upstream region. There is a tendency for a reduction in the forage and rough fishes, but the game fishes tend to increase.

The above statements give a brief description of the conditions present in a small stream that receives untreated domestic sewage. However, if the waste

---

* Published by permission of the Surgeon-General. From the Division of Public Health Methods.



FOOD CYCLE

Figure 1 - Food Cycle in a Polluted Stream. Sewage or other putrescible organic matter after entrance into a flowing stream is changed by bacterial action into ammonia and nitrites and finally into nitrates and phosphates. These latter compounds are assimilated by the algae and result in an increase in growth of these plants. The algae are consumed as food by the larger plankton, zooplankton, which in turn are eaten by fishes. Cross hatched area shows the condition of the effluent as it leaves the treatment plant. The effluent from a primary plant contains some ammonia and nitrites, but is still subject to bacterial action after disposition into the receiving stream. The degradation zone, of high bacterial action, high B.O.D. and low D.O., in the stream can be eliminated by passing the sewage through a complete or secondary treatment plant. Complete treatment converts much of the organic matter into nitrates and phosphates which become immediately available for plant growth resulting in an increased fish population.

receives complete or secondary treatment, so that the bacterial action oxidizes the sewage to available plant foods before the effluent enters the stream, the early obnoxious stage will not occur and the stream will be benefited by the fertilizing effect of the sewage for many miles of its length. Beneficial effects of primary treatment are shown by the reduction in sludge deposits and a shortening of the zone of degradation.

RELATION OF SEWAGE TO FISH LIFE

The effect of sewage on fish life varies with the season and also with the time of day. In summer the fish are active, their metabolic rate is high and more oxygen is required for their respiration than during the winter, when their activity is greatly reduced. On the other hand, during the warm summer periods the bacterial decomposition in a heavily polluted region of a stream is at its maximum, resulting in an increased biological oxygen demand and a lower dissolved oxygen concentration. The solubility of oxygen, moreover, is less in warm water than in cold water, so that less oxygen is absorbed from the atmosphere and held in solution. The increased oxygen requirement of the fish and the reduced oxygen concentration of the water renders hot weather particularly unfavorable for fish life in a polluted stream. Fish, therefore, usually die of suffocation during warm periods in regions grossly polluted by putrescible organic matter. It must not be assumed that summer is the only time that fish suffer from low oxygen concentration, because thousands of fish may die under ice by suffocation owing to the depletion of oxygen by decaying organic matter. This condition may last for only a day or two but that is sufficient time to destroy the fish population in a stream.

The toxicity of the hydrogen sulfide and other compounds produced by anaerobic bacterial action in the bottom sludge deposits may be an important factor in the death of fishes in streams receiving untreated sewage. Ellis (3) reports that 10 p.p.m. of H_2S in hard water killed goldfish in 96 hours or less. Local freshets resulting from heavy rains during low water periods tend to mix the sludge with the supernatant water and to carry the putrid mass downstream. The resulting reduction in the dissolved oxygen and the end products of anaerobic bacterial action destroy the fish for miles below.

It is also well known that heavy organic pollution causes an increase in disease, parasitism and abnormalities among fishes.

The deposition of sludge on the bottom of streams renders that portion of the stream unfit for nesting sites and will smother any eggs that may have been laid prior to the entrance of the waste. Polluted regions may act as barriers to the upstream migration of fish for the purpose of spawning.

RELATION OF ALGAE TO FISH LIFE

Algae serve directly or indirectly as food for all fishes. The green algae are the medium by which the complex organic compounds in sewage, following bacterial decomposition, are transferred to fish. The organic compounds, as previously stated, are converted by bacterial action into available plant foods. These materials are absorbed from the water by the aquatic plants and by the process of photosynthesis, and other cellular activities are converted into the living plant cell. The organic materials comprising the green algae are transferred to the fish through the medium of the zooplankton which are found associated with the algae. Small fish feed directly upon the algae and zooplankton and the adults of many species, such as the shad, live almost entirely upon the microscopic life in the water. The larger zooplankton such as Daphnia, Cyclops, etc., are important articles of diet for larval and small species of fish; in turn, these are eaten by larger fishes which may become the food of man (Fig. 1).

As stated in the first section of this paper, domestic sewage, after it has been decomposed by bacterial action, either in the stream or previously by artificial secondary treatment, increases the growth of aquatic plants by virtue of the fertilizing value of the end products. These plants furnish food for the zooplankton which in turn furnishes food for fish and thus the fish population is increased in regions where stream fertilization by sewage occurs.

Another important factor in the relation of algae to fish life is the reoxygenation of the stream by the photosynthesis of algae. The combined photosynthetic action of all the algae may increase the dissolved oxygen to supersaturation during sunny days. Purdy (4) has shown that Oocystis increases appreciably the amount of oxygen in a closed sample of water. The fact must not be overlooked, however, that the plants themselves, in addition to all forms of aquatic life, consume oxygen during the process of respiration, so the rapid rise of oxygen during the day may be followed by a disastrous fall in the early morning hours if the stream is heavily polluted by decaying organic matter.

Photosynthesis also removes from the water carbon dioxide which is produced as a waste product by the living cell and the decomposition of organic matter. Wells (5) has shown that fishes are very sensitive to small changes in the carbon dioxide content of the water and tend to avoid detrimental concentrations of this gas by moving away to more favorable locations when possible, and that fresh water species of fish ten[illegible]ct regions where the CO_2 concentration lies [illegible] 1 and 6 cc. per liter.

Turbidity may occur in hard-water ponds by the removal of the CO_2 by plants with the subsequent precipitation of the carbonates that are held in solution by the carbonic acid in the water. The removal of CO_2 tends to keep the water from becoming acid, but fish will tolerate without apparent harm a pH as low as 4.5 (6).

SUMMARY

Data obtained from a pollution survey of the Ohio River Basin clearly show that the decomposition products of domestic sewage and other putrescible organic matter increase the growth of plankton, which growth is reflected in an increase in the fish population.

Untreated or raw sewage, when in sufficient concentration, produces a toxic area below the sewer outlet. The region extends downstream for a variable distance, until the sewage is decomposed by bacteria. From this point, the stream is benefited by the fertilizing action of the decomposition products.

When the sewage has received proper secondary treatment, the toxic or degradation zone does not exist and the entire stream will be benefited biologically by the available plant foods introduced.

REFERENCES

1. F.J. Brinley and L.I. Katzin, Ohio River Pollution Survey. Report of Biological Studies. In press.
2. F.J. Brinley, Biological Studies, Ohio River Pollution Survey. I. Biological Zones in a Polluted Stream. This Journal, 14, 147-152 (1942).
3. M.M. Ellis, Detection and Measurement of Stream Pollution. Bulletin of the Bur. of Fisheries, 48, Bull. No. 22 (1937).
4. W.C. Purdy, Experimental Studies of Natural Purification in Polluted Waters. X. Reoxygenation of Polluted Waters by Microscopic Algae. U.S. Public Health Reports, 52, 29, 945-978 (1937).
5. M.M. Wells, The Reaction and Resistance of Fishes to Carbon Dioxide and Carbon Monoxide. Bull. Ill. State Lab. of Natural History, XI, Art. VIII, 557-569 (1918).
6. H.W. Brown and M.E. Jewell, Further Studies on the Fishes of an Acid Lake. Trans. Amer. Micros. Soc., 45, 20-34 (1926).

Reproduced With Permission From:
SEWAGE WORKS JOURNAL
20(1948): 292-302

BIOLOGICAL ASPECTS OF STREAM POLLUTION*

A. F. Bartsch

Senior Biologist, State Committee on Water Pollution, Madison, Wis.

The entry of pollutants into a flowing stream sets off a progressive series of physical, chemical and biological events in the downstream waters. Their nature is governed by the character and quantity of the polluting substance. Domestic or industrial effluents may adversely affect natural stream life by direct toxic action or indirectly through quantitative alterations in the character of the water or the stream bed. These facts imply that the presence of polluting substances produces physical, chemical and biological changes that may be recognized as dependable criteria of stream conditions.

The value of physical and chemical data is recognized generally by those concerned with stream pollution and its control. Methods used in gathering these data are fairly well standardized and practiced. Biological procedures have not, as yet, attained an equal degree of refinement. In some ways this is surprising, for the complex interactions resulting from stream pollution are predominantly biological. The determinations of biochemical oxygen demand and dissolved oxygen are essentially for the purpose of finding out how much bacterial food is available and how the bacteria like their diet. Other applications of these data are well known.

The biological phase of stream sanitation is still an infant science, with many of its procedures, refinements and applications still to be worked out. For this reason the following discussion is of a general nature and refers primarily to stream pollution resulting from the introduction of raw or partly treated sewage. Industrial or toxic types of wastes are not considered. Personal field observations and the publications of others have been drawn upon freely for interpretation.

Biological aspects of stream pollution will be considered in a general way from two separate but related points of view: (1) how pollutants change the character of the stream as a habitat for organisms, and (2) the action of organisms upon the pollutant and their related distribution.

The fundamentals of stream biology may best be illustrated by reference to a fictional stream whose hypothetical character may be molded with a free hand. This stream has a semi-solid bottom, medium gradient, average width of about 75 feet, and depth of 6 feet. It flows through alternating wooded and cultivated areas. It is blessed, for our purpose, by having a single source of man-made pollution -- the community of Windmill. Sewage is discharged directly to the stream.

The stream water reaching this community is not pure, for the word, as commonly used, is only relative. Drainage from the land already has added humus extracts, organic particulate matter and inorganic salts leached from the soil. Drainage from cultivated land is rich in the elements that stimulate plant growth, and pasture lands contribute organic wastes and intestinal bacteria. These contributions are sometimes called "natural pollution." Whether the origin is natural or from a sewer outlet, the stimulatory effect upon organisms is the same in principle. The recognizable result is that the unpolluted stream supports a variety of organisms as a normal biota.

---

* Presented at Twentieth Annual Convention, Central States Sewage Works Assn.; Duluth, Minn.; June 20, 1947.

EFFECTS OF POLLUTION ON BIOLOGICAL ENVIRONMENT

The entry of pollutants changes in many ways the conditions under which stream organisms normally live. This discussion can consider only a few, but there are changes in the stream bottom, in the physical and chemical properties of the water and in the competitive relations of organisms.

Sewage is a complex mixture of many kinds of substances that have been discarded by man because, to him, they have no further value. The constituents are organic and inorganic, simple compounds and complex ones. The organic substances include carbohydrates, proteins and fats as well as their decomposition products. There are salts of various kinds including ammonium salts, nitrates and nitrites. Organic growth stimulators also are a part. Some of the sewage substances are in solution, some colloidal and others suspended but capable of settling. It follows, then, that a recipient stream will have its waters affected by all fractions of the sewage while the stream bed is altered primarily by settling particulate matter.

If the stream is represented graphically (Figure 1) with mileage distances (or hours of flow) on the horizontal axis, the fictional community of Windmill is located at the zero level. Distances upstream are to the left and downstream to the right. The intensity of varying environmental conditions is plotted along the vertical axis. Food for organisms -- chemists call this B.O.D. is shown as curve F. The introduction of sewage tremendously augments the normal supply and thus alters this environmental factor. As the food supply is increased by pollution, the bacterial population tends to increase in geometric proportion and draws upon the available food (Figure 4). It is to be expected that the food supply will decline downstream and will eventually approach the pre-pollutional value. This is found to be a fact.

All organisms require oxygen for the maintenance of life. When applied to food, it functions in releasing the life-supporting energy that foods contain. Man draws upon the atmospheric supply by breathing, while most aquatic organisms draw upon the oxygen dissolved in water. Bacterial reduction of the pollutional food supply -- desirable and necessary as it may be -- is not accomplished without cost. That cost is reduction in dissolved oxygen concentration beyond the point required by desirable water animals.

The normal oxygen value of clean water is shown as areas A, from this level to 40 per cent of saturation as B, lesser concentrations as C, and those increasing beyond 40 per cent of saturation as D. These areas also mark the arbitrary limits of stream zones based upon oxygen concentration. The descriptive names are clean water for A, degradation for B, active decomposition for C and recovery for D.

If the pollutional load is fairly light or the dilution factor high, the sag curve resembles the upper curved line with normal value reestablished at 58 miles. This is accomplished by addition of oxygen from the atmosphere and through the activity of green plants. Where the content of organic matter is sufficiently high, dissolved oxygen may be reduced to zero through the oxygen-absorbing efficiency of bacteria. This is the ultimate in organic stream pollution and the circumstance upon which the following discussion is based. This poorest of stream conditions will be discussed since conditions that are less severe are then readily apparent also.

Ten miles below Windmill is the beginning of a 20-mile zone in which dissolved oxygen is absent entirely.

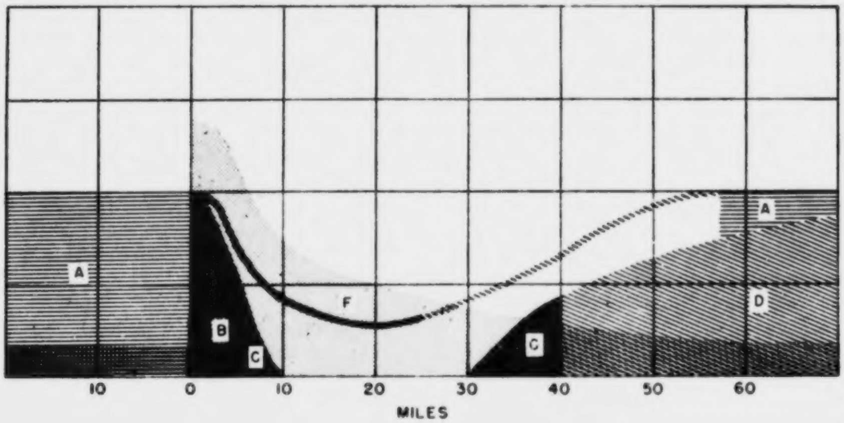


Figure 1 - Graphic representation of hypothetical polluted stream showing relationship of biotic food supply (F) and dissolved oxygen sag curve.

Here, the biotic demand for oxygen is greater than the supply provided by solution from the atmosphere. Bacteria and certain other organisms occupying this septic region are obliged to obtain the required oxygen from other sources. This they do by reducing oxygen-bearing compounds by anaerobic processes. Such activity may result in depleting the supply of oxygen found chemically in nitrates and nitrites, and reduces sulfates to hydrogen sulfide with its offensive odor and toxic action. These are some of the causes for rising gas bubbles and sludge in the septic zone. The gases alone make living conditions here unattractive for most forms of life.

Stream environment is further affected by the suspended semi-solids of sewage (Figure 2-A). These affect green free-floating stream life by immediately decreasing the transparency of the water and blotting out the sunlight. Downstream from the sewer outlet the water is turbid and slightly brownish, becoming dark and murky in the septic zone. As oxygen is added downstream by reaeration, the water gradually clears and finally is tinged with green by suspended microscopic plants.

Organisms that live in the stream bed are also affected by the suspended matters of sewage. These finally settle to the bottom (Figure 2-C) as a blanket of debris that effectively covers the normal habitat of clean water bottom life. It is an inexhaustible source of food but will sustain only those organisms that can qualify for life in that habitat. They must be efficient in obtaining oxygen, for conditions frequently are anaerobic. They must be able to burrow and creep so as to stay on top of the steadily growing layer, or else must be indifferent to being covered over. They must resist the toxic action of hydrogen sulfide and other gases that may emanate continuously from the deeper sludge layers.

Thus, it is seen that sewage alters the normal conditions of food supply, dissolved oxygen, turbidity, bottom surface, and chemical character of the stream and its bed. These are but a few of the environmental alterations that result from sewage pollution. They are sufficient to show that biological changes are sure to follow. Alteration in the competitive relations of stream life will be shown in subsequent discussion.

ACTION OF ORGANISMS UPON THE POLLUTANT AND THEIR RELATED DISTRIBUTION

It is apparent that most modern methods for the treatment of sewage depend, at some stage or another, upon the activities of living organisms. So much is this the case that all sorts of schemes have been devised for pampering the biotic associations and fostering their work. Biological competition is removed in the wastage of activated sludge, and oxygen is supplied to excess. The trickling filter brings the organisms food and oxygen and washes away their metabolic wastes and products. In the sludge digester, they are kept warm so their work proceeds properly and at a rapid pace. In the final analysis, the modern treatment plant is an artificial, telescoped, polluted stream with the zone of degradation at the primary tank and the recovery zone in the final effluent. The high efficiencies obtained are related entirely to these artificial stimulatory conditions, for the fundamental biological processes are the same as in the less efficient stream.

It has been shown that sewage is food, stimulation and habitat for simple forms of life, and that reduction in the organic stream load is accompanied by a corresponding extraction of oxygen. It is not the intent of this discussion, nor within the ability of the writer, to give in detail the precise bacterial activities involved in this accomplishment. At the same time, the basic information is important and needs consideration.

The millions of bacteria in sewage-laden waters are of a variety of kinds and have a variety of abilities. Some of these are normal inhabitants of both clean and

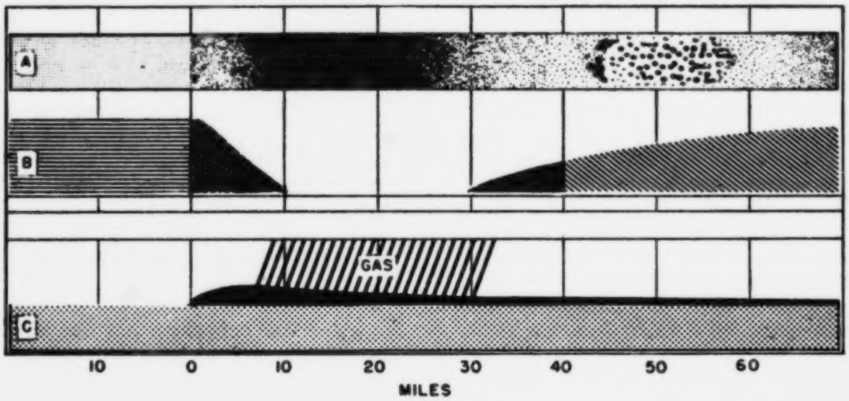


Figure 2 - Biotic habitat alterations resulting from stream pollution. (A) Physical changes in water resulting from entry of raw sewage at zero mileage level; (B) dissolved oxygen sag curve; and (C) accumulation of bottom sludge deposits and rising gas bubbles.

foul water. The presence of sewage stimulates their population increase (Figure 4). Others find their way into the stream in tremendous numbers as normal inhabitants of sewage. Some bacteria are able to multiply in the stream, while others such as B. coli and disease producers appear to die off gradually downstream. They may starve to death, be eaten by predators, be killed by high acidity, or disappear in still other unknown ways. In any event, the population peak is in the near downstream vicinity of the pollutional source.

Some bacteria can act upon a given organic compound, derive energy and growth material from it, and leave an altered residue that serves a different bacterial species or other organism in the same manner. In this way, chains of progressive actions are set in motion that result eventually in the transformation of sewage to simpler, innocuous substances. Some of these are carbon dioxide and water from carbohydrates and fats, and salts of phosphorus, sulfur and nitrogen from protein. Evidence of this mineralizing process is found in the progressive quantity shift from organic nitrogen, to ammonia, to nitrites and finally to nitrates as the water proceeds downstream.

As bacteria grow and multiply, selected constituents of the sewage are incorporated into their living substance. The ability to do this distinguishes all nonliving from living matter. It is an important ability, for in its practice a part of the sewage is set aside momentarily for action at a later time. On this account, bacteria sometimes are called concentrators of the pollutional load.

These activities of bacteria proceed in all parts of the stream. They are distributed throughout the water and are mixed into and over the bottom deposits. Under conditions of intense pollution, dissolved oxygen eventually is depleted in the flowing stream. Such depletion is more frequent and widespread in the bottom sludge. When this condition prevails, as from the 10 to 30-mile levels in the illustrative stream, bacterial action becomes of a different sort. In this septic region bacteria that are able to do so, act upon oxygen-bearing compounds in such a manner that oxygen from outside sources is not required. These bacteria are commonly called anaerobes. Their actions are to be prevented, if possible, for their products are various acids and such gases as ammonia, methane and hydrogen sulfide. Living conditions here are suitable only for organisms unaffected by these products and indifferent to oxygen supply.

Biological action in the stream continually decreases the food supply so that at 50 to 60 miles the concentration approximates the upstream values. Bacteria decrease in much the same pattern so that normal populations are attained at about the same level.

In addition to bacteria, unpolluted streams support a variety of other kinds of organisms. Those forms that produce their required food from minerals, carbon dioxide and water are members of the plant kingdom. Animals are those that require a supply of food already prepared. Bacteria and molds resemble animals in their food habits, but are classed as plants that lack the ability to make food. Organisms may be classified further by their position in the stream. Those that are small, suspended in the water and swept along with the current, are called the plankton. Plankters may be either plant or animal. Organisms that are attached to, lie upon, creep over or burrow into the stream bed are called the benthos. As has been stated, the bacteria occupy all of these positions. Large animals such as fish, frogs and turtles are not considered in this classification scheme.

Clean waters support a wide variety of organisms consisting of plant and animal plankton as well as benthic organisms. They are exacting in their habitat requirements and are affected by any interfering alterations. Normal changes in temperature, light, dissolved oxygen and food supply tend to result in shifts in the population picture. These, however, are rarely great, for predation, death and growth moderate the changing tendency and keep the biotic society in balance. In this society are organisms ordinarily associated with clean stream conditions. Some of these are game fishes such as trout, bass, blue-gills and pike, and smaller animals such as mussels, crayfish, snails and the larvae of caddisflys, stoneflies and dragon and damselflies. Shrimplike scuds may be present, swimming about on their sides or climbing over vegetation. A complete list of these organisms would be a long one.

In such a list of inhabitants would be the names of some organisms that are just holding their own, never building an appreciable population. Competition is too keen, food supply too low, and the habitat not quite suitable. Some of these would fare better in the polluted portions of the stream.

SIGNIFICANCE OF BIOLOGICAL POPULATION

The remainder of this discussion is based upon the principle that organisms differ, not only in appearance, but also in their power of response to conditions of the environment. If all moderating factors for a given organism are removed, the organism will thrive and produce tremendous numbers. On the other hand, if environmental factors are inhibitory, numbers will be small or totally absent. A set of conditions that are ideal for one organism may be lethal for another.

If changing conditions, such as pollution, are unfavorable, organisms must resist these changes, migrate or be destroyed. But, if conditions are favorable for certain organisms, these will thrive and build high populations. For this reason, the society of organisms found in zones of pollution is highly significant. It offers clues to the intensity of pollution and the degree of recovery.

Let it be supposed that the clean waters of the illustrative stream will provide suitable living quarters for a hundred different kinds of organisms, -- a balanced society of plant and animal species (Figure 3-A). With the entry of sewage, the variety decreases rapidly. Of these 100 species upstream the majority find conditions for life unsuitable in the zones of pollution. It is only downstream in the recovery zone where biotic variety makes a gain.

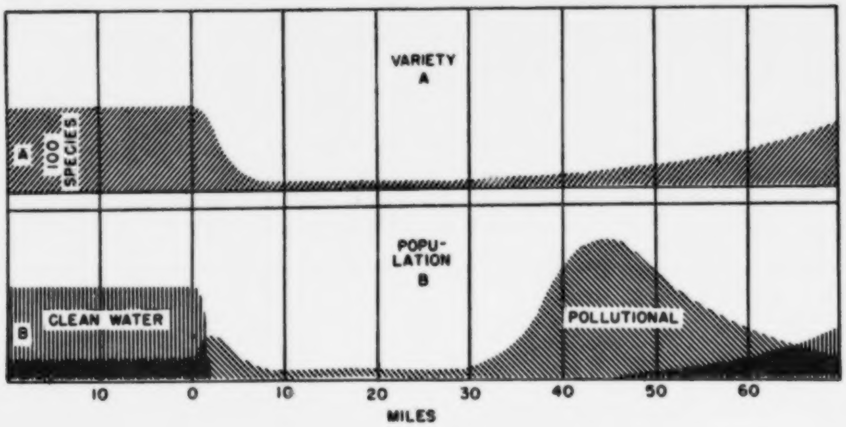


Figure 3 - Responses of bottom organisms to entry of raw sewage at zero mileage level. (A) Varity distribution; (B) population alterations of "clean water" and "pollutional" bottom forms.

Strange as it may seem, these few species in the pollutional zones find conditions quite suitable. Here they thrive in the absence of competition and with a high food supply. They are the ones that are intimately concerned with stream recovery. They may be called pollutional organisms.

If the biotic population be differentiated into clean water and pollutional organisms (Figure 3-B), their distribution may be contrasted. Bacteria are excluded from this graph. Clean water population drops abruptly to zero with the introduction of sewage. The population increases downstream with the variety increase. Pollutional population is low in clean water, but response is quick in the presence of wastes. Even these organisms decline in the true septic zone where only the anaerobes can live. The population peak will occur near the 45-mile level where food is abundant and oxygen again sufficient for the biotic needs. In the absence of a septic zone, both population peaks would roughly coincide. This condition will apply also in the following graphs. Population drop from the 45-mile point reflects approaching exhaustion of the food supply.

POLLUTIONAL ORGANISMS AND THEIR FUNCTIONS

Swimming about among the bacteria and creeping over the bottom sludge are minute animals composed of a single structural unit or cell. Some of these are able to utilize complex dissolved and particulate organic substances and in this way parallel the action of bacteria. Their prime function, however, is a more important one. They drive the bacteria and keep them at work. This is accomplished by the simple expedient of voraciously eating the bacteria so that they must reproduce to maintain their numbers. Since growth is a prelude to reproduction, biochemical oxidation proceeds at a feverish pace.

The bacteria-eaters are mainly those protozoans equipped with cilia which they use for swimming and food gathering. They move about continuously, lashing the water with their cilia and setting up currents that sweep bacteria into the gullet. This practice is carried out wherever bacteria occur. Following due process of ciliate digestion, the bacterial substance is now protozoan substance. The presence of bacteria and organic supplies results in a population peak below the bacterial peak as well as one above the septic zone (Figure 4).

But, for these protozoans all is not sublime -- they too have enemies. As they are swept downstream or flutter over the mud, they finally fall victim to rotifers, water fleas and related crustaceans that select them for variety in their diet of bacteria and small algae. The peak crustacean population is at the 58-mile point (Figure 4). And so it goes, the larger eating the smaller until the food progression leads to mussels, crayfish, small fish and large fish.

Mainly restricted to the bottom is another array of biotic forms. These are perhaps the most dependable indicators of stream condition. Ordinarily, the pollutional bottom is a confusion of biological activity -- each member of the assemblage going about his own business of gathering food and reproducing. To them, stream recovery is merely incidental. The distribution of species is governed by the stringency of habitat conditions.

Rat-tail maggots (Figure 5-A) are a sign of extremely poor conditions. Thus ugly larva of the drone-fly (Eristalis tenax) lies buried in the mud with the tail extended to the surface for air. For this reason, dissolved oxygen is not a consideration and it may penetrate into the septic zone.

Next in line come the sludge-worms (Tubificidae) (Figure 5-B), reddish in color, 1/2 inch to 1-1/2 inch

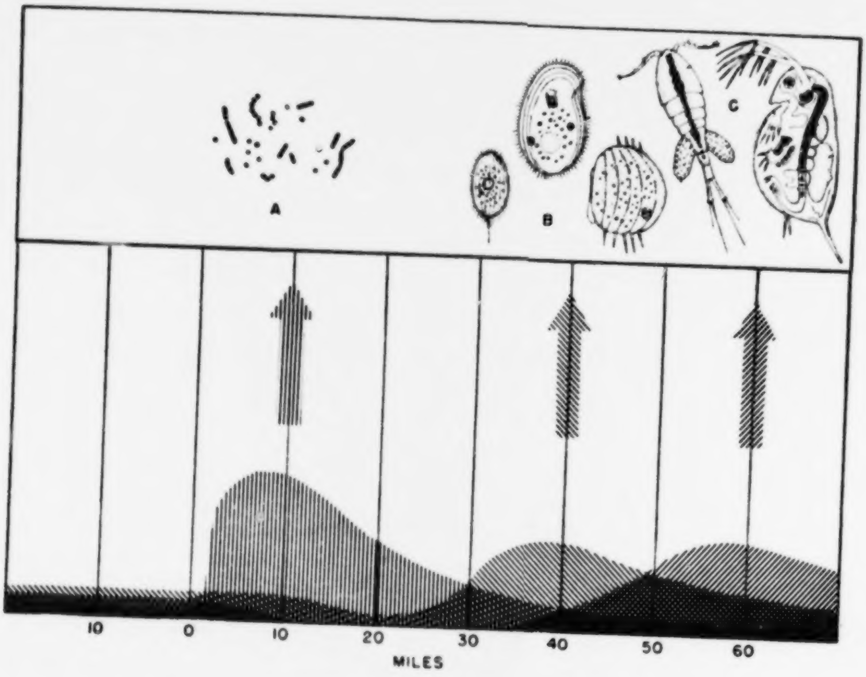


Figure 4 - Linear alterations in populations of bacteria, ciliate protozoans, and crustaceans.

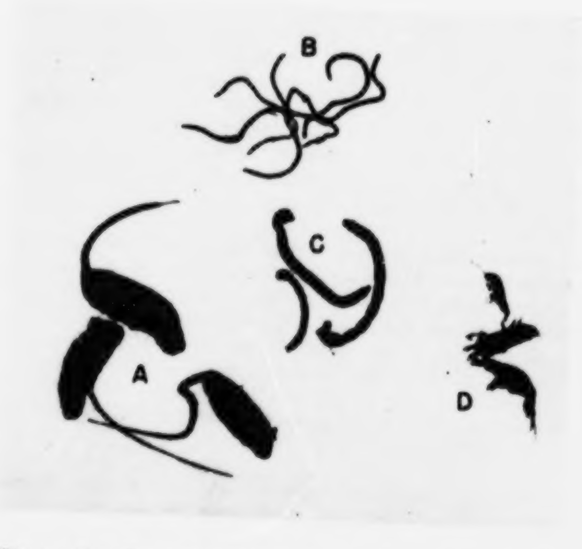


Figure 5 - Bottom organisms found in zones of pollution. (A) Rat-tail maggot (Eristalis tenax); (B) Sludge-worm (Tubifex sp.); (C) Blood-worm (Chironomus sp.); and (D) Sow-bug (Asellus communis). Approximately natural size.

in length. They burrow in the mud where organic content is high. They excavate in the upper layers of the sludge, passing large quantities through the intestinal tract and straining out the food. With the posterior part of the body projecting into the water, the worms cast rejected parts of the sludge on the surface in the form of fecal pellets. The work accomplished in this manner is tremendous. The sludge is worked over, perforated and its organic content reduced.

Frequently these worms are so numerous that the stream bed appears as a red undulating sheet. They occupy the zones of degradation, active decomposition and the upper part of the recovery zone. They are absent from the septic region.

Blood-worms (Chironomus sp.) also are burrowers in the mud. These are red, jointed, worm-like animals (Figure 5-C) that eventually transform to midge-flies. In this larval stage, they occupy burrow-like tubes constructed of sludge stuck together with an adhesive substance. Empty tubes are common and may occur in heaps. Their food habits are similar to the sludge worms, but they are more exacting in their habitat requirements. For this reason they reach their peak in the recovery zone.

At this point, also, the sow-bug or water-log-louse (Asellus communis) makes its first appearance. They are flattened, greyish animals about 1/4 inch long

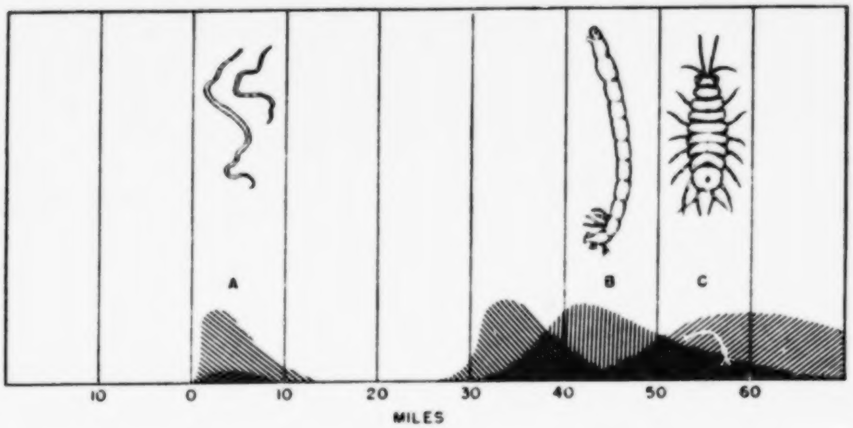


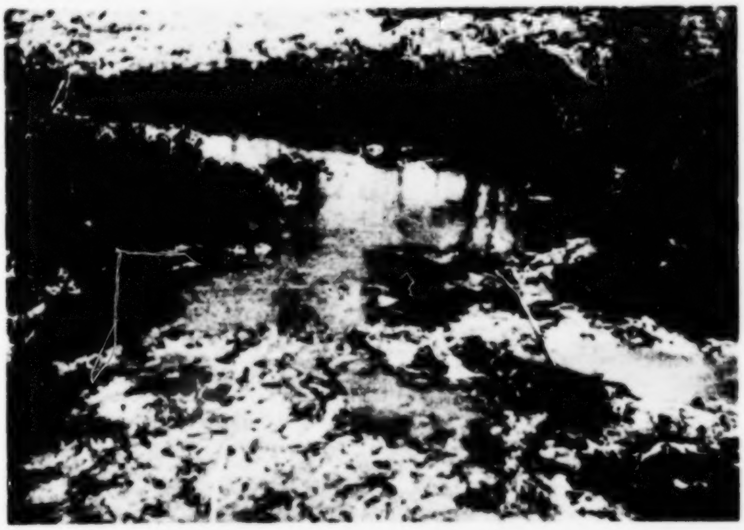
Figure 6 - Linear alterations in populations of sludge-worms (A), blood-worms (B), and sow-bugs (C).

(Figure 5-D), related to the scuds found in clean water. They are provided with jointed appendages, of which six pairs are modified as legs. They crawl about on the bottom, under stones, or climb among water weeds. They do not move by swimming.

Sow-bugs are omnivorous in their feeding habits but seem to prefer dead and decaying vegetable matter. Their oxygen requirements apparently are greater than those of sludge-worms or blood-worms. They are common in the recovery zone where dissolved oxygen in the supernatant water exceeds 40 per cent of saturation. They indicate improving conditions.

If the distribution of sludge-worms, blood-worms and sow-bugs are plotted together, their population peaks occur in the succession shown in Figure 6.

In addition to bacteria, other plants also are involved in stream recovery. Sewage molds and filamentous bacteria may be seen attached to sticks, stones and vegetation, waving gracefully in the current (Figure 7). They function with the bacteria in biochemical oxidation. They are whitish gray, becoming tinged with yellow, red or brown when old. In the zone of degradation, growth is widespread and luxuriant. It persists to the septic region and reappears feebly with



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 7 - Sewage mold (Sphaerotilus natans) attached to sticks, stones and vegetation and waving in the current.

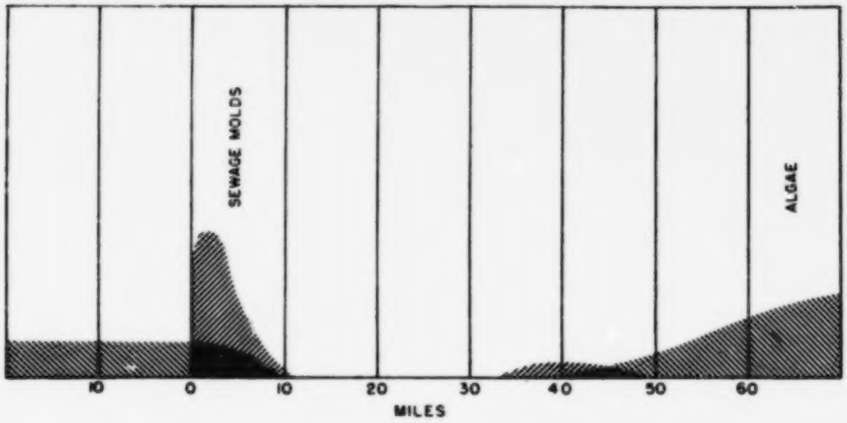


Figure 8 - Linear alterations in populations of sewage molds and plankton algae.

oxygen restoration. Its presence is a reliable index of intense pollution -- especially by carbohydrates.

The distribution of plankton algae, as a group, is in contrast to the sewage molds (Figure 8). The clean water population is reduced rapidly in the degradation zone where turbidity is high. They may be very sparse or absent throughout the septic region and then increase to a new high under the stimulatory influence of phosphorous and nitrogen compounds oxidized from sewage sources.

In the pollutional zones benthic algae may occur as a dark film over the bottom or as a bright green scum along the banks and in quiet spots. Most of these are filamentous, although some may be single-celled and able to swim. Some, at least, absorb certain organic solutions from the water. Valuable as this may be, the algae are more valuable for the oxygen they add to the water in the process of making food. They are instrumental in drawing up the lower end of the sag curve. They supply oxygen for the biochemical demand and speed the recovery process.

SUMMARY

Living organisms are affected by the conditions of stream pollution. Their distribution is altered and may be used to complete the pollutional picture obtained by the usual testing procedures. Their activities contribute tremendously to stream recovery by using pollutants as a source of energy and growth material. Some eat bacteria and thus accelerate their biochemical activities. Green organisms supply oxygen so greatly needed for B.O.D. satisfaction and sag curve elevation. Metabolic wastes, products and dead bodies are passed back to the stream as an altered link of a continuous chain. Biological efficiency in the stream falls far short of that in the treatment plant. A good treatment plant at Windmill would confine these activities and restore utility to the running stream.

Reproduced With Permission From:

PURDUE UNIVERSITY ENGINEERING BULLETIN -
PROCEEDINGS OF THE 8th INDUSTRIAL WASTE CONFERENCE
1953: 295-316

SOME IMPORTANT BIOLOGICAL EFFECTS OF POLLUTION OFTEN DISREGARDED IN STREAM SURVEYS

Clarence M. Tarzwell and Arden R. Gaufin

Chief and Biologist - Biology Section
Environmental Health Center, Public Health Service
Cincinnati, Ohio

The complexity of the pollution problem is being constantly intensified by the ever-increasing variety of pollutants that are added to streams. Due to the fact that pollutants, domestic and industrial, represent only a portion of the many factors which determine stream environments, the same pollutant may not bring about similar conditions in different streams. The character of the watershed, including soil type, amount and type of ground cover, and land uses; the amount, seasonal distribution, and type of precipitation; the frequency of floods and the amount of erosion; and the character of the stream banks, bottom materials, gradient and stream flow are all of importance. These and other factors determine stream characteristics, environmental conditions, the aquatic biota, and in large part the effects of different polluting substances.

SOME BIOLOGICAL EFFECTS OF POLLUTANTS

Pollutants may alter the stream environments and thereby affect aquatic life in a number of ways. These environmental changes may include an increase in stream temperatures; changes in the character of the stream bottom; increase in turbidity; changes in the content of dissolved oxygen; increase in dissolved nutrients; production of undesirable growths; deposition of sludge beds; and the addition of toxic wastes. The degree or extent of the effect of these changes on aquatic life varies with the type and amount of the pollutant and the character of the receiving water. It is the purpose of this paper, therefore, to point out some of the possible effects of pollution on aquatic life and to indicate pertinent ecological conditions which should be noted in stream surveys.

Water used for cooling purposes in industrial processes may become so hot and be of such quantity that it may substantially raise the temperature of the receiving stream. The addition of a waste or wash water having a fairly constant temperature tends to stabilize stream temperatures, especially during the winter (31) and may increase productivity through increased metabolic activity. Moderate heating by increasing metabolism can hasten the natural purification process and shorten the pollutional zones (56). In trout streams even a slight rise in temperature is usually undesirable (6). Due to the removal of shade and other factors (73) (64), many trout streams are approaching borderline temperatures (87) and a rise of even two to three degrees Fahrenheit sometimes is sufficient to eliminate trout or to make the stream less favorable for them (6). If temperatures become too warm for trout on only one day in the year, that stream ceases to be a trout stream (47). Highest stream temperatures during the day usually occur between 2 and 4 p.m. and peak temperatures usually occur after a succession of warm days and nights. Few trout, even the most tolerant species, can survive temperatures above 82 to 83 degrees Fahrenheit even for very short periods (44) (24). During the summer of 1930, the senior author found rainbow trout living in the South Branch of the Pere Marquette river in Michigan at a peak temperature of 83 degrees Fahrenheit. This temperature occurred for a short time between 3 and 4 p.m. During July, 1931, brook trout survived peak temperatures of 81 and 82 degrees Fahrenheit in the East Branch of the Black river in Michigan.

Studies of trout streams in many portions of the country and observations on the effects of the removal of shade and the raising of stream temperatures has lead the senior author to conclude that water temperatures need not be raised to lethal levels in order to affect trout populations adversely. When stream temperatures are raised so that they consistently exceed 70 degrees Fahrenheit in summer, environmental conditions become less favorable for the cold water species and more favorable for the warm water forms (6). Due to this change in environmental conditions, minnows, suckers, and other warm water fishes may increase in numbers at the expense of the Salmonoids (87) which decrease in numbers to such an extent that sometimes they represent less than ten percent of the total fish population (43) (69).

Fish population studies conducted by the senior author in 1931 and 1935 in the East Branch of the Black river in northern Michigan, indicated that trout comprised 9.6 percent of the total number of fishes and 8.6 percent of the weight of the total population of fishes. Minnows, however, accounted for over 60 percent of the total number of fishes taken and made up 9.6 percent of the total weight of the population. Suckers comprised 23.3 percent of the total number of fishes and 66.7 percent of the weight of all fishes. Studies carried out in 1934 on another warm trout stream, the Pigeon river, indicated that the trout comprised 14.3 to 19.7 percent of the total population; whereas minnows comprised 58 to 68 percent of the population. Fish population studies made during the same period on a cold stream, the West Branch of the Sturgeon river, which lies near to and parallels the Pigeon river, indicated that in the latter stream trout comprised 93.8 to 98.7 percent of the total number of fishes and over 99 percent of the total weight of the population. Shetter and Hazzard (67) found that in the lower portion of the South Branch of the Pine river of Michigan, where the water is fairly warm, trout comprised 13.6 percent of the population, while in a cold stream, the Little Manistee river, they comprise 64 to 91 percent of the population.

In streams inhabited by the warm water species, sunfishes, white bass, black bass, crappie, etc., a slight rise in temperature may increase productivity (78) (33). Temperatures directly lethal to fishes are not so likely to occur in such streams. In the northern portion of the country, bass have been killed by water temperatures of 94 degrees F. (Michigan Lake 1936). In the TVA Reservoirs of northern Alabama water temperatures sometimes reach 96 degrees F. without apparent harmful effects to bass and other native fishes. Near Savannah, Georgia, all fish in a shallow pond died in water which reached 108 degrees F.

The type of bottom material directly affects the productivity of a stream. Shifting sand bottom streams are virtually aquatic deserts (74) while rubble gravel bottoms usually support large populations of aquatic insects (75) (76) (77). The addition of sand, clay, or other inorganic wastes which covers more productive bottom types is, therefore, detrimental to the overall productivity of a stream.

Inert inorganic wastes may be added to streams from a number of sources such as hydraulic and placer mining operations (68), mine tailings, gravel pit washings (89), etc. However, the greatest and most widespread sources of this type of pollutant is soil erosion (7) (17). During the past century floods and soil erosion have been greatly increased in some areas by deforestation (5) (14), fire, overgrazing (30) (41), and ill-advised agricultural practices (34) (86). Materials eroded from the watersheds and washed into streams affect the aquatic environments in a number of ways (48). Sand and silt fill pools, destroy fish cover and spawning beds (35), and cover productive bottom types (45) (46) (52). Erosion and eroded materials have converted good fish streams into wide washes where the low water flow meanders over the wide bottom in a thin sheet or disappears completely in the deposits which choke the former stream channel.

Eroded materials also cause turbidity which affects productivity and water uses. Turbidity decreases light penetration and thereby limits the growth of phytoplankton and other aquatic plants which are of outstanding importance as a basic food for aquatic animals and as a producer of oxygen by photosynthesis (49). The photosynthetic activity of aquatic plants plays an important part in stream reaeration and in the natural purification process (10) (55) (60). Although turbidity prevents or limits algal growth, it does not eliminate the bacterial action which mineralizes organic wastes (13). Thus, turbid waters may transport the bi-products of bacterial action on organic wastes and the effluents of sewage treatment plants considerable distances before they are utilized (83). When the water clears due to impoundment or other causes so that the phytoplankton can grow, these fertilizing materials are utilized and may produce troublesome blooms, or taste and odor problems far from the source of pollution.

Soil washings from eroded areas are usually infertile and generally reduce productivity by choking or covering densely populated rubble gravel riffles, and rich bottom deposits. Washings, from fertile areas, where accelerated erosion is just beginning, or from rich well-fertilized agricultural areas, carry a great deal of nutrient materials into lakes and streams and increase productivity. This fertilizing effect may be so great that nuisance blooms of algae may develop each year such as those that occur in many Iowa lakes. These blooms become especially troublesome when domestic sewage is also added to the water (9) (40). Further, in some areas, blooms of toxic algae are frequent and severe (8) (50).

The sole detrimental effect of putrescible wastes is often considered to be oxygen depletion. Putrescible wastes, however, may affect environmental conditions, aquatic life, and water uses in a variety of ways. Organic wastes serve as nutrients which stimulate growth and reproduction of aquatic life (10). The first group to be stimulated are the bacteria which are chiefly responsible for the decomposition and conversion of organic wastes into nutrient materials such as nitrate, phosphate and carbon dioxide (16). If the organic materials occur in sufficient concentration the bacteria may utilize nearly all of the dissolved oxygen and produce conditions which are unfavorable for many other forms of life (9). When such conditions prevail the zone of greatest bacterial growth is designated as a septic zone, in which the species of macro-invertebrates are limited to those organisms that have the ability to live under low oxygen concentrations and those which have adaptations for breathing atmospheric oxygen (3) (31). Although the number of different species of macro-invertebrates occurring in the septic zone, is only a fraction of the number found in the other well-recognized zones of pollution, productivity from the standpoint of numbers and volume of organisms produced is several times that of the other zones (4) (12) (31) (63). This is well-demonstrated in Figure 1 which shows the number of different species and the number and volume of organisms found per unit area under summer conditions in the various pollutional zones of Lytle creek, a small stream near Cincinnati, Ohio, which has been intensively studied by the Public Health Service. This small number of species and very large number of individuals constitute a biological indication of septic conditions (59).

The decomposition of organic wastes by the bacteria converts them into materials such as carbon dioxide, nitrate, and phosphate, which are readily used by phytoplankton. As these materials become available,

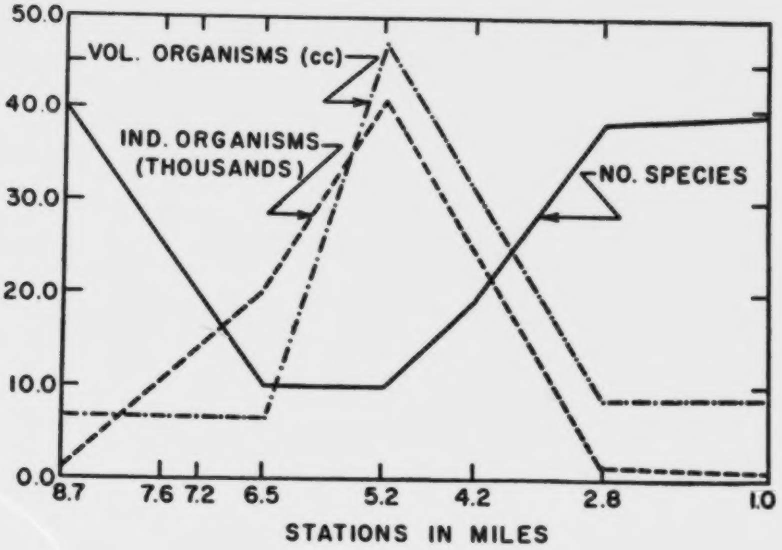


Figure 1 - Macro-invertebrate distribution, Lytle creek, summer conditions.

there is a gradual buildup of phytoplankton in the lower septic zone which reaches its peak in the recovery zone (28). Considerable oxygen is produced by the phytoplankton when conditions are favorable (60). Investigations carried out at the Cincinnati station of the Public Health Service have demonstrated that in many small and moderate sized streams which are fairly clear, reoxygenation by photosynthesis completely overshadows reoxygenation by aeration. Because of the large amounts of food present in the recovery zone of streams polluted with putrescible wastes, phytoplankton populations may become very large and dissolved oxygen levels frequently may exceed saturation by 100 percent (31) (56) (60) (61). In fact, a superabundance of dissolved oxygen may be an indication of organic pollution.

Because photosynthetic activity is dependent upon sunlight there may be wide variations in dissolved oxygen in a polluted stream during the 24-hour daily cycle (31) (38) (60) (66) (70) (84). Dissolved oxygen concentrations are usually highest between 2 and 4 p.m. and are lowest just before or after sunrise. At one station on Lytle creek, dissolved oxygen varied from a high of 19.4 p.p.m. in the afternoon to a low of 0.7 p.p.m. the next morning. The Lytle creek investigations showed that these daily variations in dissolved oxygen are greatest in the late spring and summer seasons and that the difference between maximum and minimum levels is most marked in the recovery zone. Nocturnal-dirunal variations in dissolved oxygen in Lytle creek at different seasons of the year are shown in Figures 2 and 3. It is apparent from these graphs that the season greatly influences minimum oxygen levels.

Year-round studies in Lytle creek and the Great Miami river have indicated a fairly definite seasonal cycle of environmental conditions (31). During the winter months there was an abundance of dissolved oxygen throughout the streams with levels at no time falling far below saturation. During spring as the water becomes warmer, oxygen was depleted just below pollution outfalls during the early morning hours. As the season advanced this oxygenless area increased in extent and duration until in late summer there was a definite septic zone in which oxygen was absent or essentially absent at all times. In Lytle creek there was an almost equal stretch of stream just below this zone in which oxygen was absent or very low during the night. As the fall season advanced the oxygenless zone decreased in extent and duration until by the beginning of winter there was an abundance of oxygen throughout the stream. Character of flow is of great importance in determining the seasonal pattern of stream conditions. If flows are extremely low in winter, septic conditions may persist into December, while if they are high they may not develop in summer.

It will be noted in Figures 2 and 3 that the greatest variations in dissolved oxygen occurred during the spring and summer in the zone of recovery and that concentrations of dissolved oxygen reached a maximum in that zone. These conditions prevailed because, first, phytoplankton growth and photosynthesis reached a peak in the recovery zone, and second, under conditions of supersaturation, riffles and general stream turbulence in the clean water zone brought about the release of oxygen rather than the absorption of additional amounts.

The decomposition of putrescible wastes either by natural purification or by sewage treatment provides a supply of those materials which stimulate the growth of large populations of phytoplankton (10) (28) (91). These algal populations through photosynthetic action aerate the stream and at times produce supersatura-

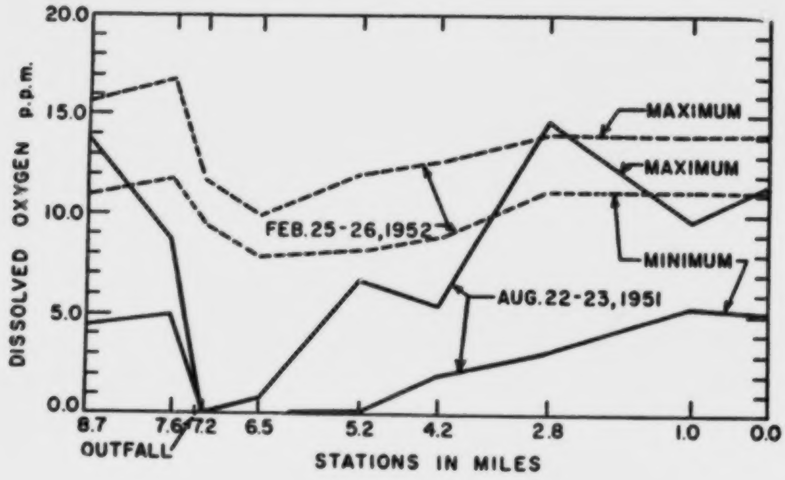


Figure 2 - Range in dissolved oxygen, Lytle creek.

tion (31) (49) (84). There is, however, a point, not definitely known as yet, beyond which the algae cease to be beneficial and may actually be harmful. This is due to the fact that at levels of supersaturation photosynthetic oxygen in streams is rather rapidly released to the atmosphere and this rate of release increases in proportion to the rate of production. Further, while the phytoplankton may produce large quantities of oxygen during the day (61), it also uses oxygen for respiration at all times and during the night excessively large phytoplankton populations may deplete the dissolved oxygen and cause fish kills (49) (54). Such a fish kill occurred in Lytle creek in the fall of 1952. At that time due to extremely low flows of clear water the nutrients in the stream caused an excessive phytoplankton bloom which produced dissolved oxygen levels in excess of 21 p.p.m. in the afternoon but during the night reduced dissolved oxygen levels to such an extent that a severe fish kill occurred in the recovery and clean water zones. It is possible, therefore, for a large secondary sewage treatment plant, located on a small to moderate sized stream, to indirectly cause fish kills through the production of nutrients which bring about excessive growths of algae which in turn deplete the oxygen at night by their respiration and decay.

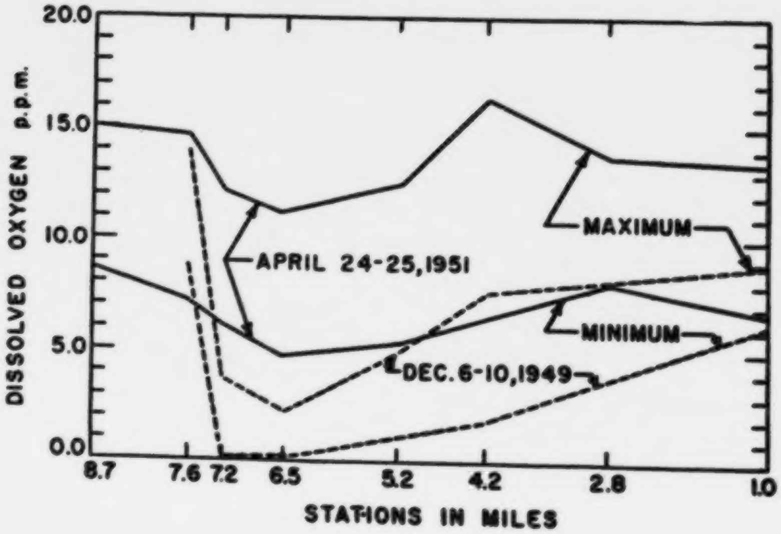


Figure 3 - Range in dissolved oxygen, Lytle creek.

These algal blooms may have other undesirable effects. The algal growths may cause tastes and odors in water supplies farther downstream (93), they may clog filters, or they may create toxic conditions at points of concentration (26) (49) (51) (92). The constant addition of even low levels of nitrogen and phorphorus, no greater than those which may occur naturally, can greatly stimulate algal growths (32). Under natural conditions available phosphates and nitrogen are rather rapidly utilized and bound up in the bodies of the phytoplankton. Studies made by Wiebe (personal communication) in Norris Reservoir, Tennessee, for example, indicated that all of the available phosphorus was utilized in the upper half of the reservoir. In reservoirs, nutrients are also removed by the dying and settling out of the plankters (90). If there are periodic floods which bring in silt that covers the dead organisms which have settled to the bottom, fertility is permanently removed from the reservoir. However, if they are not covered with silt and stratification occurs in the reservoir, they may be decomposed by bacterial action and the nutrient materials may be recirculated throughout the reservoir by the spring and fall overturns. In lakes which are stratified, this is considered to be a basic cause of the spring and fall peaks in plankton growths (90). Reservoirs may, therefore, remove or create problems, depending on conditions. Their mode of operation also influences downstream conditions (83).

In streams severely polluted with organic wastes the stream bottom in the septic zone is usually covered with grayish growths commonly referred to as "sewage fungus" (58) (91). These growths are often regarded as being a result of oxygen deficiencies because they are generally limited to septic areas during the season in which stream surveys are most often conducted. It has been shown, however, that these prolific growths are not produced by oxygen deficiencies but by concentrations of organic matter, chiefly nitrogenous and carbohydrate material (11). The low oxygen concentrations usually associated with them are incidental and the result of the decomposition of the organic material upon which they are dependent for existence. The common designation of these growths as "sewage fungus" is somewhat unfortunate because, while they may contain some fungi such as Geotrichum, Leptomitas, and Fusarium, they are often chiefly composed of the bacteria Sphaerotilus, Zoogloea, and Beggiatoa, and certain ciliated protozoans such as Vorticella, and Carchesium (11) (59). Taken collectively these organisms constitute a pollutional blanket which influences natural purification and other stream life in such ways as the breaking of the natural food cycle in the stream and creating an unfavorable bottom condition.

The year round studies which have been carried out on Lytle creek have demonstrated that when floods are not too severe during the winter months, there is a downstream extension of this pollutional blanket. This is brought about by a change in environment conditions which are more favorable for such growths; namely, an increase in the organic content in the water in the lower zones (36). This increase of organic matter further downstream in winter is believed to be chiefly due to two factors, first, a reduction in the time of flow to about one-fifth that of the summer period due to larger flows, and second, to low water temperatures which decrease metabolic activity of the bacteria and thus the rate of decomposition of the organic matter. The down stream extension of the pollutional blanket covers the bottom and alters the habitat so that it is unfavorable to most of the macro aquatic invertebrates which normally occur in the recovery and upper clean water zones, with the result that they must migrate or die. Its direct effect on the larger forms was well illustrated by the fate of stoneflies, mayflies, caddis flies, and other insects which were washed into polluted sections of Lytle creek. These insects soon became so covered with growths that they were overwhelmed and smothered. The accumulations of these growths on some mayflies, stoneflies, dragonflies, and other insects which were taken under such conditions are shown in Figures 4 and 5. In these figures normal insects are included to enable comparison with those which have been covered with the growths. The pollutional blanket does not develop if severe floods occur during the winter period. It develops during periods of normal or low water and it is removed by the first flood. After its removal the area is practically barren of bottom life and some time is required for it to be repopulated.

Seining studies demonstrated that fish were forced downstream during the period of existence of the pollutional blanket. In fact, although dissolved oxygen concentrations were near saturation throughout Lytle creek during the winter months the fishless area was about twice as long as it was in the summer months. This movement downstream of the fish population was probably due to the destruction of their normal food by the pollutional blanket. It is evident, therefore, that oxygen depletion is not the only factor responsible for the creation of fishless areas in streams polluted with organic wastes.

In instances of organic pollution it is generally assumed that the critically low oxygen levels which occur in streams in late summer during periods of low flow and high temperatures most seriously affect the overall economy of the stream. While this is generally true it may not always be the case. Observations have indicated that in small streams under such conditions increased metabolic activity plus very slow flows have resulted in greatly shortened septic zones. Further, if the water is clear, reaeration due to the photosynthetic activity of the phytoplankton builds up oxygen levels in the recovery zone such that the fishless zone is at a minimum. In addition, low water and slack current allows the settling out of a great deal of material so that it is for the time being removed from the stream. However, the first real rise in stream flow usually picks up these sludge beds and may create a real problem. Studies on the Great Miami river indicate that the first high water after an extended low water period picks up sludge beds and carries them farther downstream with a resultant critical decrease in dissolved oxygen over a more extensive area than was previously affected. The harmful effects of the removal of accumulated sludge beds have been noted by several investigators (9) (59) (88). Forbes (27) noted fish kills in the Illinois and Rock rivers due to the flushing out of sludge beds

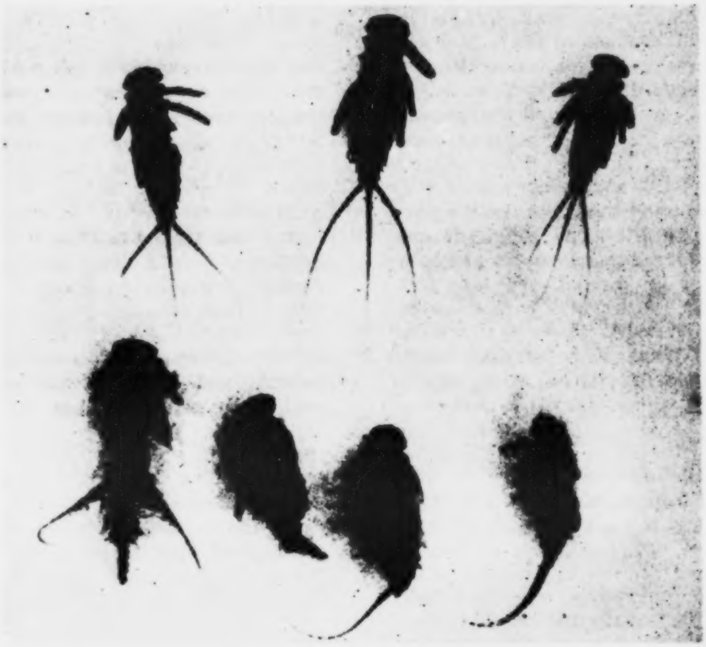
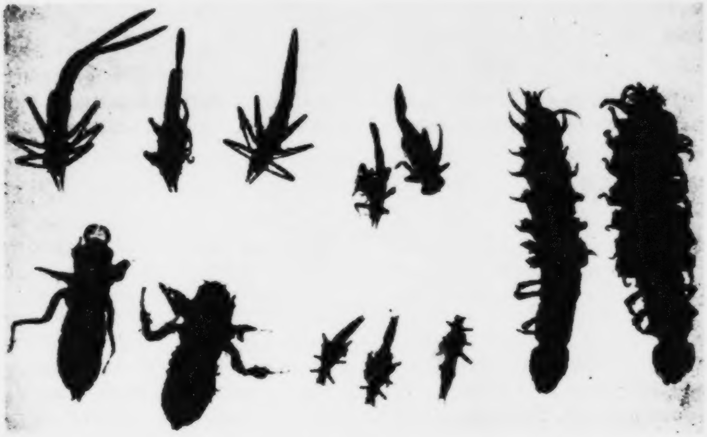


Figure 4 - Upper row, normal mayflies. Lower row, those which have been covered with "sewage fungus" (bacteria and protozoa).



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 5 - Growths which develop on aquatic insects in polluted streams due to the downstream extension of the pollutional blanket (sewage fungus). Normal specimens are included for comparison. It can be noted how the gills are covered by the growth.

which had accumulated during periods of low water. The removal of settleable solids is, therefore, of value for the protection of stream life.

Toxic wastes may eliminate the fish population in certain areas or they may decimate only certain species or certain developmental stages. Thus, adults may migrate into an area and live where reproduction is not successful. Some fish food organisms are more sensitive to certain toxic materials than are fish (1). In such instances fish populations may be reduced due to lack of food without actually killing the fish. In addition to destroying fish, toxic wastes may interfere with the natural purification process by limiting those organisms which break down the wastes. If these wastes are to be controlled, their mode of action and their influence on aquatic life must be considered (19) (20) (22). Materials toxic to aquatic life can most readily be detected and their strength estimated by means of bioassays (21) (23).

The character of the water and its mineral content can alter considerably the toxic effects of a given chemical or waste (21). For example, an acid waste added to a highly alkaline stream may be much less toxic than it is when added to an acid stream (18). The reverse is true for alkaline wastes. Further, ammonia salts are much more toxic to fish at high pH levels while copper and other heavy metals are more toxic in soft acid waters. Antagonism and synergy are also of great importance. For example, it has been demonstrated that when salts of copper and zinc occur together they are several times as toxic as when they occur separately at comparable concentrations (84). It is evident, therefore, that the toxicity of wastes cannot be represented in chemical terms alone but should be combined with data secured by means of bioassays.

DISCUSSION

The foregoing has served to indicate that various types of pollutants may have a variety of effects on streams and their biota. Environmental conditions, which largely govern the natural purification process, vary widely in different streams. The capacity to assimilate and purify wastes and the rate of purification, therefore, is not a constant for all streams. Surveys designed to evaluate pollutional conditions and the ability of a stream to assimilate wastes must give full consideration to these environmental conditions.

While dissolved oxygen is of great importance it is only one of a complex of factors which constitute the aquatic environment. The species composition of the aquatic population in a given area is determined by the environmental conditions which have prevailed during the developmental period of the organisms involved (61). If at any time during its development, environmental conditions become lethal for a given organism, that organism will be eliminated even though the unfavorable conditions are of very short duration (9) (47). The aquatic population which occurs in a given area is, therefore, a representation or indicator of environmental conditions which have prevailed during the life history of the organisms comprising the population (3) (15) (53) (63). It is this property of indicating past environmental conditions, especially the extreme conditions of brief duration, that make aquatic populations such valuable indicators of pollution (25) (31) (42). In using biological indicators, however, single species do not possess high index value. Our Studies have indicated that it is the qualitative and quantitative composition of the population which is of importance in denoting past conditions. Further, the absence of clean water species is much more significant than the presence of tolerant species (31) (62).

Data on fish populations are especially valuable for indicating pollutional conditions because fish are the chief end product of the aquatic cycle. Data on the qualitative and quantitative composition of fish populations, rate of growth, average size, and catch per unit effort of the sport and commercial fishery are especially valuable for denoting the suitability of water conditions and the economic and recreational losses due to pollution. In fact the suitability of a water for fish life is best defined by its productivity.

Pollution affects fish populations in a number of ways depending upon the nature and concentration of the waste. Moderate amounts of organic materials which do not seriously affect dissolved oxygen levels, may serve as nutrient materials and increase fish production. This occurred in the Illinois river prior to 1920 (29).

Periodic fish kills due to spills attract a great deal of attention but they are, in general, much less harmful than the slow gradual increase in pollution which slowly decimates the population in such a way that the dead fish, or the decline in the fish population, are not noted and the fishery is destroyed without exciting public protest. In some streams in the east fisheries values have been gradually reduced over such a long period that the fishery potentials are not now generally realized (39).

Although extensive areas of streams are often made fishless the effects of pollution are not always on an all-or-none basis (62). The complete absence of fish is usually common information, but deteriorations in the quality of the population is not generally apparent without some sampling studies. In polluted streams the game fishes may be reduced in number or eliminated while the coarse species or those most tolerant of low oxygen concentrations comprise the remaining population (37). It has been the senior author's experience that when coarse fishes become abundant, they crowd out the game fishes which results in a marked decline in the sport fishery because the coarse fishes are not desired by the sportsmen and are inferior as food fishes (79) (80) (81) (82).

A somewhat routine procedure has grown up and been adopted for pollutional surveys. In these surveys, very often most of the effort is devoted to measurements of the discharge from various plants; to routine bacteriological, physical, and chemical studies of selected effluents; and to a compilation of existing data on domestic and industrial water uses, stream flows, sources of pollution, and sewage treatment. The physical, chemical, and bacteriological studies on the pollutants being discharged into the stream usually include determinations for D.O., B.O.D., pH, temperature, turbidity, settleable solids, total alkalinity, the coliform index, and others depending upon the nature of the waste discharged (2).

Sampling in the receiving stream is usually limited in scope and carried out during the period of low flow and high temperatures. It is customarily confined to the taking of grab samples at selected stations which are often highway bridges. Determinations made on these stream samples are generally the same as those made on the pollutional effluents.

All these studies can be worthwhile and are essential in many instances but their adequacy and value for meeting the overall problem would be greatly increased if some additional studies were made and a somewhat different approach were adopted. The collection, abstracting, rearranging, and assembling of existing data, the routine collection and analysis of samples, and the use of empirical constants and formulae may lead to a "cook book" approach to the overall pollution problem and the concept of the stream as a biological entity is lost.

Further, the routine application of the customary survey procedures can result in wasted time and effort. An example is the routine determination of B.O.D.'s and the coliform index for a waste, the detrimental effect of which is toxicity. Repeatedly, survey groups which go into the field to investigate one of the common biological effects of pollution, a fish kill, follow the customary chemical approach and carry into the field only equipment for the collection and analysis of water samples. Ordinarily no biological observations or studies are made. Further, when samples of the supposedly offending waste are brought into the laboratory an indirect approach is customarily used. First consideration is usually given to B.O.D. or oxygen consumed tests. When the possibility of toxicity is considered and the highly toxic materials, for which routine methods of analyses have been developed, are not found, little thought is given to the probability of the presence of those highly toxic materials which are not readily separated and measured chemically (19). If some of the more toxic substances are indicated by the analyses, their toxicity to aquatic life is frequently estimated on the basis of a limited knowledge of their toxicity in simple solution. Consideration is not given to the fact that the quality of the receiving water greatly influences their toxicity, or that they occur not alone, but in mixtures, and their action may be greatly modified by antagonism and synergy.

The simplest and most direct approach for determining toxicity which takes into consideration all these factors is to make a bioassay of the waste in question, using local species of fish as reagents and employing the receiving water for dilution of the waste. When bioassays are combined with chemical studies for toxicity determinations, much more progress will be made toward detecting, analyzing, and meeting toxic waste problems.

Considerable attention has been devoted to the development of procedures for estimating the dissolved oxygen levels that may be present at critical times of the year or during periods of recorded low flows. Customarily, the data on which these calculations are based are secured from grab samples taken without regard to photosynthetic activity and diurnal variations in dissolved oxygen (72) (85). When collecting data from the calculation of the sag curve attention should be directed not only to variations in water level, rate of flow, temperatures, and the character of the stream, but also to photosynthetic activity and the time of day during which samples are taken. From the standpoint of the protection of fish life, averages of dissolved oxygen concentrations determined from grab samples, are of little value and may be actually misleading unless the determinations on which the averages are based are taken at the correct times and minimal levels are known. It is the extreme and not the average conditions which are important.

Grab samples taken without reference to daily fluctuations in D.O. concentrations or the zones of pollution do not give an adequate or accurate measure of oxygen conditions in a stream. Further, in the calculation of sag curves, photosynthetic activity is neglected (71), even though in many streams it completely overshadows reaeration from the atmosphere and is at its peak during periods of low flow and clear water. In addition, much still remains to be learned concerning the effects of sludge deposits and the relation between the breaking down of the wastes under the controlled conditions in the B.O.D. test and what actually occurs in the stream. It has been shown that all wastes are not broken down at the same rate and that nitrification begins before the fifth day (65). The problem is not simply the amount of pollution discharged. It involves the fate of the waste materials in the stream under varying seasonal conditions, the capacity of the stream for handling that particular waste, and the effects on aquatic life of recreational and economic importance.

If environmental changes brought about in a stream due to pollution cannot be observed or measured, there is justification for estimating them inferentially. However, since the toxicity of wastes to fish life can be determined directly by bioassays, the direct effects of pollution on the aquatic biota can be determined by observation and population studies. Because the aquatic biota present in a stream or stream section serves to indicate past environmental or pollutional conditions, it is evident that the inferential approach is not always necessary. Since acceptable dissolved oxygen concentrations and regulations governing the discharge of toxic wastes are frequently set up to meet the requirements for fish life, and because the natural purification of putrescible wastes in streams is a biological process, the necessity of biological studies to supply pertinent information and to supplement and strengthen the customary stream investigations is apparent.

ACKNOWLEDGMENTS

The biological surveys and investigations upon which much of this paper is based have required the active assistance and cooperation of several individuals. The authors wish to express their appreciation for assistance rendered to the following: C.M. Palmer, Peter Doudoroff, Max Katz, Thomas E. Maloney, George Paine, Harold Walter, and Charles Howard.

BIBLIOGRAPHY

1. Anderson, Bertil G., "The Toxicity Thresholds of Various Substances Found in Industrial Wastes as Determined by the Use of Daphnia magna." Sewage Works Journal, Vol. 16, pp. 1156-1165 (1944).

2. Anonymous. "Lower Platte River Basin Water Pollution Investigations." U.S.P.H.S., Division of Water Pollution Control, Missouri Drainage Basin, Kansas City, Missouri, pp. 1-184, (1950).

3. Baker, Frank C., "The Molluscan Fauna of the Big Vermillion River Illinois." Illinois Biol. Monographs, Vol. 8, No. 2, (1922).

4. Bartsch, A.F., "Biological Aspects of Stream Pollution." Sewage Works Journal, Vol. 20, pp. 292-302, (1948).

5. Bates, C.G. and A.J. Henry, "Forest and Stream Flow Experiment at Wagon Wheel Gap, Colorado." U.S. Mo. Weather Rev., Sup. 30, pp. 1-79, (1928).

6. Belding, D.L., "Water Temperature and Fish Life." Trans. Am. Fish. Soc., Vol. 58, pp. 98-105, (1928).

7. Bennett, H.H., "Soil Erosion Studies." U.S. Dept. of Agr. Yearbook 1934, pp. 321-323, (1934).

8. Brandenburg, T.O. and F.M. Shigley, " 'Water Bloom' as a Cause of Poisoning in Livestock in North Dakota." Jour. Amer. Vet. Med. Assoc., Vol. 110, p. 384, (1947).

9. Brinley F.J., "Biological Ohio River Pollution Survey. I. Biological Zones in a Polluted Stream. II. Plankton Algae As an Indicator of the Sanitary Condition of a Stream" Sewage Works Jour., Vol. 14, pp. 147-159, (1942).

10. Brinley, F.J., "Sewage, Algae, and Fish." Sewage Works Jour., Vol. 15, pp. 1139-1152, (1943).

11. Butcher, R.W., "Contributions to Our Knowledge of the Ecology of Sewage Fungus." Trans. British Mycological Soc., Vol. 17, pp 112-124, (1932).

12. Butcher, R.W., "The Biological Detection of Pollution." Inst. of Sewage Purification. Paper presented at a meeting of the Midland Branch, Birmingham, Eng., pp. 3-8 (1946).

13. Butterfield, C.T., "Some Functions of the Bacteria in the Purification of Polluted Water." Jour. Bact., Vol. 39, pp. 527-533, (1940).

14. Church, J.E., "Restraining Effects of Forests on Sudden Melting of Snow." Eng. Rec., Vol. 69, No. 24, p. 674, (1914).

15. Claassen, P.W., "The Biology of Stream Pollution." Sewage Works Jour., Vol. 4, No. 1, pp. 165-172, (1932).

16. Crohurst, H.R. and W.C. Purdy, "Disposal of Sewage in the Potomac River." Senate Doc. No. 172, 72nd Congress, 2nd Session, 65 pp., (1933).

17. Dana, S.T., "Farms, Forests, and Erosion." U.S. Dept. Agri-Yearbook 1916, pp. 107-134, (1916).

18. Doudoroff, Peter and Max Katz, "Critical Review of Literature on the Toxicity of Industrial Wastes and Their Components to Fish. I. Alkalies, Acids, and Inorganic Gases." Sewage and Ind. Wastes, Vol. 22, pp. 1432-1458, (1950).

19. Doudoroff, Peter, "Biological Observations and Toxicity Bio-assays in the Control of Industrial Waste Disposal." Proc. Sixth Ind. Waste Conf., Purdue Univ. Eng. Ext. Bull. No. 76, Vol. 35, No. 6, pp. 88-104, (1951).

20. Doudoroff, P., B.G. Anderson, G.E. Burdick, P.S. Galtsoff, W.B. Hart, R. Patrick, E.R. Strong, E. W. Surber, and W.M. Van Horn, "Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish." Sewage and Industrial Wastes, Vol. 23, No. 11, pp. 1380-1397, (1951).

21. Doudoroff, Peter, "Some Recent Developments in the Study of Toxic Industrial Wastes." Proc. Fourth Ann. Ind. Waste Conf. at State College, Wash., pp. 21-25, (1952).

22. Ellis, M.M., "Detection and Measurement of Stream Pollution." Bull. No. 22, U.S. Bureau of Fish, Bull. Bur. Fish., Vol. 48, pp. 365-437, (1937).

23. Ellis, M.M., B.A. Westfall, and M.D. Ellis, "Determination of Water Quality." Research Rep. No. 9, U. S. Fish and Wildlife Ser., 122 pp., (1946).

24. Embody, G.C., "Concerning High Water Temperatures and Trout." Trans. Am. Fish. Soc., Vol. 51, pp. 58-64, (1921).

25. Farrell, M.A., "A Biological Survey of the St. Lawrence Watershed. IX. Studies of the bottom fauna in Polluted Areas." Biological Survey No. 5, Supp. Twentieth Ann. Rept., pp. 192-197, (1930).

26. Fitch, C.P., L.M. Bishop, et al., " 'Water Bloom' as a Cause of Poisoning in Domestic Animals." Cornell Vet., Vol. 24, No. 1, pp. 30-39, (1934).

27. Forbes, Stephen A., "Definite Results of Survey Work on the Illinois River." Trans. Am. Fish. Soc., Vol. 41, pp. 75-89, (1912).

28. Forbes, S.A. and R.E. Richardson, "Studies on the Biology of the Upper Illinois River." Bull. Ill. State Lab. of Nat. Hist., Vol. 9, Art. 10, (1913).

29. Forbes, S.A. and R.E. Richardson, "Some Recent Changes in Illinois River Biology." Bull. Ill. Nat. Hist. Sur., Vol. 13, Art 6, (1919).

30. Forsling, C.L., "A Study of the Influence of Herbaceous Plant Cover on Surface Run-Off and Soil Erosion in Relation to Grazing on the Wasatch Plateau in Utah." U.S. Dept. Agri. Tech. Bull. 220, (1931).

31. Gaufin, A.R. and C.M. Tarzwell, "Aquatic Invertebrates as Indicators of Stream Pollution." Public Health Reports, Vol. 67, No. 1, pp. 57-64, (1952).

32. Hasler, A.D., "Eutrophication of Lakes by Domestic Drainage." Ecology, Vol. 28, pp. 383-395, (1947).

33. Hathaway, E.S., "The Relation of Temperature to the Quantity of Food Consumed by Fishes." Ecology, Vol. 8, No. 4, pp. 428-434, (1927).

34. Hendrickson, B.H., "The Chocking of Pore-Space in the Soil and Its Relation to Runoff and Erosion." Trans. 15th Ann. Amer. Geophy. Union. pp. 500-505, (1934).

35. Henshall, James A., "Concerning the Protection of Fish, Fish Food, and Inland Waters." Trans. Am. Fish. Soc., Vol. 49, No. 3, pp. 141-147, (1920).

36. Hoskins, J.K., "Studies of Natural Purification in the Illinois River." Eng. News-Record, Vol. 89, pp. 1078-1079, (1922).

37. Katz, Max and Arden R. Gaufin, "The Effects of Sewage Pollution on the Fish Population of a Midwestern Stream." Trans. Am. Fish. Soc., Vol. 82, pp. 156-165, (1953).

38. Kehr, R.W., W.C. Purdy, J.B. Lackey, O.R. Placak, and W.E. Burns, "A Study of the Pollution and Natural Purification of the Scioto River." Public Health Bull., No. 276, (1941).

39. Kendall, William Converse, "The Status of Fish Culture in our Inland Public Waters and the Role of Investigation in the Maintenance of Fish Resources." Roosevelt Wildlife Bull., Vol. 2, No. 3, pp. 204-351, (1924).

40. Lackey, James B., "Stream Microbiology." Stream Sanitation by Earle B. Phelps. John Wiley and Sons, New York, Chapter 7, pp. 227-265, (1944).

41. Loudermilk, W.C., "The Role of Vegetation in Erosion Control and Water Conservation." Jour. Forestry, Vol. 32, No. 5, pp. 529-536, (1934).

42. Moore, Emmeline, "Stream Pollution and Its Affects on Fish Life." Sewage Works Jour., Vol. 4, p. 159, (1932).

43. Moore, Emmeline, J.R. Greeley, C.W. Greene, H.M. Faigenbaum, F.R. Nevin, and H.K. Townes, "A Problem in Trout Stream Management" Trans. Am. Fish. Soc., Vol. 64, pp. 68-80, (1934).

44. M'Gonigle, R.H., "Algae, a Factor in Some Hatchery Mortalities." Trans. Am. Fish. Soc., Vol. 64, pp. 416-423, (1934).

45. Needham, Paul R., "A Quantitative Study of the Fish Food Supply in Selected Areas." Supp. 17th Ann. Rept., (1927). Dept. Cons., J.B. Lynn, Albany, New York, pp. 192-208, (1928).

46. Needham, Paul R., "Quantitative Studies of Stream Bottom Foods." Trans. Am. Fish. Soc., Vol. 64, pp. 238-247, (1934).

47. Needham, Paul R., "Trout Streams." Comstock Publishing Company, Inc., Ithaca, New York, 233 pp., (1938).

48. Nevin, James, "Changing Food Conditions of the Trout Family." Trans. Am. Fish. Soc., Vol. 49, pp. 29-32, (1920).

49. Olson, Theodore A., "Some Observations on the Interrelationship of Sunlight, Aquatic Plant Life, and Fishes." Trans. Am. Fish. Soc., Vol. 62, pp. 278-289, (1932).

50. Olson, Theodore A., "History of Toxic Plankton and Associated Phenomena." Sewage Works Eng., Vol. 20, No. 2, p. 71, (1949).

51. Olson, Theodore A., "Toxic Plankton." Water and Sewage Works. Vol. 99, pp. 75-77, (1952).

52. Pate, V.S.L., "Studies of Fish Food in Selected Areas." Biological Survey of the Upper Hudson Watershed. Supp. to 22nd Ann. Rept., N.Y. State Cons. Dept., pp. 130-156, (1932).

53. Patrick, Ruth, "Biological Measure of Stream Conditions." Sewage and Industrial Wastes, Vol. 22, pp. 926-938, (1950).

54. Prescott, G.W., "Objectionable Algae With Reference to the Killing of Fish and Other Animals." Hydrobiologia, Vol. 1, No. 1, pp. 1-13, (1948).

55. Purdy, W.C., "Potomac Plankton and Environmental Factors." Hygienic Lab. Bull., No. 104, pp. 130-191, (1916).

56. Purdy, W.C., "Study of the Pollution and Natural Purification of the Ohio River. Part I. The Plankton and Related Organisms." Pub. Health Bull., No. 131, pp. 1-78, (1922).

57. Purdy, W.C., "Biology of Polluted Waters." Jour. Am. Water Works Assoc., Vol. 16, pp. 45-54, (1926).

58. Purdy, W.C., "Activities of Plankton in the Natural Purification of Polluted Water." Am. Jour. Public Health, Vol. 18, pp. 468-475, (1928).

59. Purdy, W.C., "Influence of the Discharge of Sewage on Minute Forms of Stream Life." 6th Ohio Conf. on Sewage Treatment, pp. 21-34, (1932).

60. Purdy, W.C., "Results of Algal Activity, Some Familiar, Others Obscure." Jour. Am. Water Works Assoc., Vol. 27, pp. 1120-1133, (1935).

61. Purdy, W.C., "Experimental Studies of Natural Purification in Polluted Waters. Part 10. Reoxygenation of Polluted Waters by Microscopic Algae." Public Health Rep., Vol. 52, pp. 945-978, (1937).

62. Richardson, Robert E., "Changes in the Bottom and Shore Fauna of the Middle Illinois River and Its Connecting Lakes Since 1913-1915 as a Result of the Increase Southward of Sewage Pollution." Bull. Ill. Nat. Hist. Sur., Vol. 14, Art. 4, (1921).

63. Richardson, Robert E., "The Bottom Fauna of the Middle Illinois River 1913-1923. Its Distribution Abundance Valuation and Index Value in the Study of Stream Pollution." Ill. Nat. Hist. Sur. Bull., Vol. 17, Art. 12, (1928).

64. Roth, Filibert, "The Fisherman and Reforestation." Trans. Am. Fish. Soc., Vol. 13, pp. 164-168, (1906).

65. Ruchhoft, C.C., O.R. Placak, and M.B. Ettinger, "Correction of B.O.D. Velocity Constants for Nitrification." Sewage Works Jour., Vol. 20, pp. 832-840, (1948).

66. Schroepfer, George J., "An Analysis of Stream Pollution and Stream Standards." Sewage Works Journal, Vol. 14, No. 5, pp. 1030-1063, (1942).

67. Shetter, David S. and Albert S. Hazzard, "Species Composition by Age Groups and Stability of Fish Populations in Sections of Three Michigan Trout Streams During the Summer of 1937." Trans. Am. Fish. Soc., Vol. 68, pp. 281-302, (1939).

68. Smith, Osgood R., "Placer Mining Silt and Its Relation to Salmon and Trout on the Pacific Coast." Trans Am. Fish. Soc., Vol. 69, pp. 225-230, (1940).

69. Smith, Lloyd L., Jr., Raymond E. Johnson, and Laurence Hiner, "Fish Populations in Some Minnesota Trout Streams." Trans. Am. Fish. Soc., Vol. 76, pp. 204-214, (1949).

70. Stone, A.R., and W.E. Abbott, "Diurnal Variations in the Dissolved Oxygen Content of Polluted Water." Water and San. Eng., Vol. 1, pp. 33-35, (1951).

71. Streeter, H.W., "The Rate of Atmospheric Reaeration of Sewage Polluted Streams." Pub. Health Rep., Vol. 41, pp. 247-262, (1926).

72. Streeter, H.W., "Measures of Natural Oxidation in Polluted Streams. II. The Reaeration Factor and Oxygen Balance." Sewage Works Journal, Vol. 7, pp. 534-552, (1935).

73. Surber, Thaddeus, "Biological Surveys and Investigations in Minnesota." Trans. Am. Fish. Soc., Vol. 52, pp. 225-238, (1922).

74. Tarzwell, Clarence M., "Trout Stream Improvement in Michigan." Trans. Am. Fish. Soc., Vol. 61, pp. 48-57, (1931).

75. Tarzwell, Clarence M., "Experimental Evidence as to the Value of Trout Stream Improvement in Michigan." Trans. Am. Fish. Soc., Vol. 66, pp. 177-187, (1937).

76. Tarzwell, Clarence M., "Factors Influencing Fish Food and Fish Production in Southwestern Streams." Trans. Am. Fish. Soc., Vol. 67, pp. 246-255, (1938).

77. Tarzwell, Clarence M., "An Evaluation of the Methods and Results of Stream Improvement in the Southwest." Trans. Third No. Am. Wildlife Conf., pp. 339-364, (1938).

78. Tarzwell, Clarence M., "The Fish Population in a Small Pond in North Alabama." Trans. Fifth No. Am. Wildlife Conf., pp. 245-251, (1940).

79. Tarzwell, Clarence M., "A Second Season of Creel Census in Four Tennessee Valley Authority Reservoirs." Trans. Sixth No. Am. Wildlife Conf., pp. 202-221, (1941).

80. Tarzwell, Clarence M., "Fish Population in the Backwater of Wheeler Reservoir and Suggestions for Their Management." Trans. Am. Fish. Soc., Vol. 71, pp. 201-214, (1942).

81. Tarzwell, Clarence M., "Valley Needs Commercial Fishery." Alabama Conservation, Vol. 14, No. 10, p. 7, (1943).

82. Tarzwell, Clarence M., "The Possibilities of a Commercial Fishery in the TVA Impoundments and Its Value in Solving the Sport and Rough Fish Problem." Trans. Am. Fish. Soc., Vol. 73, pp. 137-157, (1945).

83. Tarzwell, C.M. and C.M. Palmer, "Ecology of Significant Organisms in Surface Water Supplies." Jour. Amer. Water Works Assn., Vol. 43, No. 7, (1951).

84. Tarzwell, Clarence M. and Peter Doudoroff, "Applications of Biological Research for the Control of Industrial Wastes." Proc. Nat. Tech. Task. Comm. on Ind. Wastes, Cincinnati, Ohio, June 3-4, 1952, pp. 1-18, (1952).

85. Theriault, E.J., "The Rate of Deoxygenation of Polluted Waters." Pub. Health Rep., Vol. 41, pp. 207-217, (1926).

86. Thompson, W.T., "Is Irrigation Detrimental to Trout Culture?" Trans. Am. Fish. Soc., Vol. 41, pp. 103-114, (1912).

87. Titcomb, John W., "Forests in Relation to Fresh Water Fishes." Trans. Am. Fish. Soc., Vol. 56, pp. 122-129, (1926).

88. Trautman, Milton B., "The General Effects of Pollution on Ohio Fish Life." Trans. Am. Fish. Soc., Vol. 63, pp. 69-72, (1933).

89. Viosca, Percy, "Water Pollution in Louisiana." Trans. Am. Fish. Soc., Vol. 56, pp. 101-107, (1926).

90. Welch, Paul S., Limnology. 471 pp. McGraw Hill Book Co., N.Y., (1935).

91. Weston, R.S. and C.E. Turner, "Studies on the Digestion of a Sewage Filter Effluent by a Small and Otherwise Unpolluted Stream." Mass. Inst. Tech., Sanitary Research Lab., and Sewage Exp. Sta., Vol. 10, pp. 1-43, (1917).

92. Wheeler, R.E., J.B. Lackey, and S. Schott, "A Contribution on the Toxicity of Algae." Pub. Health Rep., Vol. 57, No. 45, pp. 1695-1701, (1942).

93. Whipple, G.C., The Microscopy of Drinking Water (Revised by G.M. Fair and M.C. Whipple). 586 pp. John Wiley and Sons, N.Y., (1948).

Reproduced With Permission From:
BIOLOGICAL PROBLEMS IN WATER POLLUTION, pp 144-163, U.S. PUBLIC HEALTH SERVICE,
ROBERT A. TAFT SANITARY ENGINEERING CENTER, CINCINNATI, OHIO. 1957

BIOLOGICAL INDICES OF WATER POLLUTION WITH SPECIAL REFERENCE TO FISH POPULATIONS*

Peter Doudoroff
U. S. Public Health Service

and

Charles E. Warren

Department of Fish and Game Management
Oregon State College, Corvallis, Oregon

A number of investigators have very recently published discussions having to do with biological indices and biological measures of water pollution (1) (2) (7) (13) (14) (15) (16) (26) (27) (28) (29) (30) (36) (38). Fjerdingstad (12) has discussed some of the pertinent European literature. The fundamental concepts presented by these authors are not original, for the idea that aquatic organisms can be useful "indicators" of environmental conditions, and particularly of the degree of pollution of water with organic wastes, has a long history (12). Because of certain novel features and the relatively wide scope of the studies, and the broad implications of some of the conclusions, the work of Patrick (26) (27) (28) (29) (30) has attracted much attention in the United States and seems to deserve the closest scrutiny.

Although much has been written about the various biological indices, there has been no general agreement among the authors as to the meaning of some of the most important terms used in this literature and little effort to clarify the terminology. In view of the variety of backgrounds and dominant interests of individuals concerned with waste disposal and with the effects of wastes on receiving streams, it is not surprising that the term "pollution" does not have exactly the same meaning for all. It is regrettable that a variety of meanings have come to be associated with technical terms such as "biological indicator of pollution". Some of the differences of opinion as to what the biological indices are and what may be their utility doubtless stem from a lack of agreement on the meaning of the word "pollution". Investigators proposing the use of different indicators of pollution should have clarified, it would seem, their ideas as to just what constitutes pollution, or, in other words, exactly what it is that the indicators can be expected to indicate. Too often this has not been done, or the ideas and definitions presented have not been carefully developed and appear to be unsound from a practical standpoint.

Should the mere change (physical, chemical, or biological) of some aquatic environment resulting from waste disposal be regarded as pollution even when ordinary human use and enjoyment of the water and of associated natural resources have not been affected adversely? When there is evidence of environmental change, is this always reliable evidence of damage to a valuable natural resource? May not certain beneficial uses of water be sometimes seriously interfered with by the introduction of wastes which may cause little or no detectable alteration of biological communities? Have there been any studies which have conclusively demonstrated a useable fixed relation between the biological indices of pollution and the actual fate or change in value of aquatic resources which are subject to damage by pollution? If water pollution can be the result of introduction of any of a great variety of substances, organic and inorganic, is it proper to refer to those biotic responses which are only known to occur in the presence of putrescible organic wastes (i.e. to organic enrichment of water) as "indices of pollution"? Can there be any general biological solution for all problems of detection and measurement of water pollution, or is effort being wasted in a search for such a general solution? Are broad limnological investigations being undertaken where intensive study and appraisal of supposedly damaged natural resources of obvious value to man would be more profitable? Is immediate practical value of research results being claimed improperly in an effort to justify fundamental limnological studies for which no such justification should be necessary? These are questions which all biologists interested in water pollution should perhaps ask themselves. Many of these questions have no categorical answer, but it is hoped that the following discussion will prove thought-provoking. It may not only call attention to certain inconsistencies in claims made and terminology used, but may also indicate the need for revision of objectives or a change of emphasis in pertinent future investigations.

Biological investigation now is an integral part of water pollution detection and control, and biologists have become increasingly aware of their opportunities for contributing to progress in this field of work. Their ideas have been solicited and have been well received by other specialists. In trying to aid the advancement of their science, biologists owe it to their profession to seek thorough understanding of the practical problems of water pollution control. Understanding the complexity of these problems will make apparent the need for thorough and critical testing of new ideas previous to their widespread practical application.

---

* Miscellaneous Paper No. 31, Oregon Agricultural Experiment Station.

First, it is necessary to consider the meaning of the term "pollution". The introduction of any foreign substance which merely alters the natural quality of water without materially interfering with any likely use of the water cannot be said in a practical sense to constitute pollution. Virtually every stream and lake in any inhabited region receives at least a trace of something which measurably or not measurably alters the natural quality of the water. What is significant or important from a practical standpoint is not the mere presence of the added material, but its influence upon the economic and esthetic value of the water, or on human welfare in a broad sense. It appears that most authorities in the field of water pollution control and abatement agree in defining water pollution as an impairment of the suitability of water for any beneficial human use, actual or potential, by any foreign material added thereto.

This definition agrees with repeatedly expressed judicial opinion, that is, with definitions of "pollution" and of "clean water" established by courts of law. The following legal definition, cited on page 100 of "Water Quality Criteria", a publication of the California State Water Pollution Control Board (4) is typical: "For the purposes of this case, the word 'pollution' means an impairment, with attendant injury, to the use of water that plaintiffs are entitled to make. Unless the introduction of extraneous matter so unfavorably affects such use, the condition created is short of pollution. In reality, the thing forbidden is the injury. The quantity introduced is immaterial." Other definitions cited agree essentially with this one.

In accordance with the above definition of the word pollution, a demonstrable change of some components of the biota of a stream clearly caused by the discharge of some waste into the water is not invariably evidence of pollution, any more than is a demonstrable chemical change. If it cannot be reasonably asserted that a hazard to human health or interference with some beneficial use of the stream such as fishing, must accompany a particular alteration of the biota, the change cannot correctly be said to indicate pollution. Even the discharge of a waste which eliminates virtually all organisms initially present in a very small or temporary stream capable of supporting no aquatic life of any value to man is not necessarily pollution. Oxygen-depleting organic wastes may be thoroughly mineralized in such streams through natural self-purification processes, so that only harmless substances and beneficial plant nutrients may reach larger watercourses to which these streams are tributary.

In agreement with the definition offered above, Beck (1) has defined pollution broadly as "the alteration of any body of water, by man, to such a degree that said body of water loses any of its value as a natural resource."

Patrick (28), on the other hand, has proposed a distinctly different, strictly biological definition. This author defines pollution as "any thing which brings about a reduction in the diversity of aquatic life and eventually destroys the balance of life in a stream." By way of explanation, it is further stated that "As conservationists interested in using rivers today - but not abusing them so that they are damaged in the future - this is the basis on which pollution should be judged. For it is by preserving the biodynamic cycle that the ability of a river to rejuvenate itself is maintained."

Unfortunately it is not clear just what is to be regarded as pollution according to the definition given by Patrick. Is any reduction in the diversity of aquatic life evidence of pollution which will eventually destroy the "balance of life", or only such a severe reduction of the diversity of life that the ability of the stream to "rejuvenate itself" is indeed destroyed? A reduction of species numbers is not always necessarily followed by the eventual destruction of the "balance of life" in a stream and of the ability of the stream to "rejuvenate itself" (i.e., to undergo natural self-purification). Patrick (28) has pointed out that the so-called "food chain" in aquatic environments "consists of many series of interlocking links so that if one series is broken another can take over so that the chain is not destroyed." It is well known, also, that in certain "zones" of streams heavily and continually enriched with organic wastes relatively few animal and plant species are present, as a rule, yet natural purification proceeds at a very rapid rate. Here, as in an efficient trickling filter, an ideally adapted and obviously vigorous, healthy, and in certain respects very well balanced biota of limited variety can exist, and the organic waste is mineralized far more rapidly and efficiently than it could possibly be in a previously uncontaminated stream with its original, primitive biota. The ability of the stream to "rejuvenate itself" certainly cannot be said to have been destroyed, or even impaired.

Thus, a stream can be seriously polluted, in any usual sense of the word, without lasting destruction of the "balance of life" and of self-purification capacity (which balance hardly can be permanent anyway, in any unstable environment). On the other hand, mere reduction of the diversity of aquatic life without impairment of any important "food chain" (i.e., the food supply of valuable fishes, etc.), or interference with existing stream uses, does not necessarily have anything to do with the conservation of natural resources. It appears, therefore, that the last-mentioned definition of pollution is unsatisfactory, from a practical standpoint, no matter how it was meant to be interpreted.

Careful consideration of the other pertinent writings of Patrick and of the proposed method of judging stream conditions leads to the conclusion that probably this author regards any marked reduction of the diversity of aquatic life as evidence of pollution.

Beck (1) states that "Patrick's methods suggest that the bio-dynamic cycle should be maintained in the primitive condition," allowing for no equitable stream use, for "any deviation from the primitive bio-dynamic cycle is interpreted by Patrick as evidence of pollution." Actually Patrick has not suggested that an entirely primitive condition of every stream biota should be maintained and has classified as "healthy" certain stream sections which evidently were not in the primitive state. A diversity of organisms approaching that found under undisturbed or primitive conditions does seem to have been regarded, however, as being characteristic of all "healthy", unpolluted waters. This interpretation of Patrick's views may be right or wrong. In any case, the need for clarification thereof, and for better agreement among biologists as to the meaning of terms too often loosely used, is apparent. It is noteworthy that Patrick's definition of pollution, quoted above, implies that an alteration of water quality cannot be pollution if it has no appreciable effect on the diversity of aquatic life, and it can be interpreted as meaning that a marked reduction of the diversity of aquatic life is always associated with pollutional abuse of the aquatic environment. Probably few if any workers directly concerned with water pollution abatement or control can approve such a definition.

One can hardly maintain that the relative worth of any biological environment depends on the number of species that it supports, rather than on the relative abundance of species of some importance or value to man. The presence of many different weeds does not usually contribute to the value of a pasture. Also, it is not always correct to assume that any marked modification of a natural environment and of its original, primitive biota will result in their economic degradation, that is, a reduction in value. The clearing, irrigation, and cultivation of desert and other almost worthless lands, the application of agricultural and other poisons for the control of various pests and weeds, and many other human activities can, indeed, greatly enhance the value of the affected lands while drastically modifying their biotas and reducing the numbers of species present. Not only the production of valuable crops is thus promoted, but sometimes also the production of equally valuable wild game. On the other hand, the destruction of only one or a few animal or plant species of outstanding value (e.g., by some selective poison) obviously can mean great loss. This loss is in no way ameliorated by the fact that most of the organisms in the same environment are not noticeably affected. It is evident that a change of any biota considered as a whole (e.g., the number of species represented, etc.) may not be a direct nor always reliable index and measure of damage to any valuable natural resource. There seems to be no sound basis for a general assumption of their strict or even approximate parallelism.

Although most authors evidently have recognized the economic significance of pollution, it appears that when devising their biological indices and measures of water pollution and its severity some biologists have completely disregarded all economic considerations. They seem to have curiously attached at least as much importance to the elimination of any species of diatom, protozoan, rotifer, or insect as to the disappearance of the most valuable food or game fish species. Yet, some have claimed that their measure of the harmful effects of pollution is a direct measure and therefore is more reliable than any chemical evidence or measure of pollution. Why the fate of harmless algal, protozoan or insect species can be said to indicate directly the extent of damage to a valuable fish population or to any commercial, recreational, or other use of water has not been explained.

If biological indices and measures of the severity of pollution cannot be relied upon always to reveal even the extent of damage to valuable aquatic life, they certainly do not indicate accurately the general pollutional status of any water. Water which is rendered biologically sterile by addition of some substances such as chlorine, or is appreciably enriched with some organic wastes, other than domestic sewage, may be of good sanitary quality and suitable for most ordinary domestic, agricultural, and industrial uses. On the other hand, water in which aquatic life is not markedly and adversely affected can be contaminated with dangerous pathogens or with chemicals which may seriously interfere with one or more of the abovementioned uses. In view of the great variety of water uses, and the number and complexity of considerations (physical, chemical, biological, psychological, economic, and sociological) which evidently must enter into any reliable determination of the degree of interference with these uses by pollution, the evaluation of the over-all pollutional damage cannot be a simple matter. Any contention that some biological observations alone can cut across all of this complexity and show clearly whether the actual and potential uses of a stream have or have not been affected, and the magnitude of the total damage, would appear to be an oversimplification of the problem. It must be admitted that probably nobody has come forth yet with a clear statement of this claim. An yet, unless a different meaning is made perfectly clear, is not this claim implicit in every asseration to the effect that a generally applicable and reliable biological index or measure of the pollutional status or condition of streams has been devised and developed?

Biotic responses to all of the numerous and very different water pollutants are not alike. Early students of water pollution (23) (24) (31) dealt chiefly with pollution by putrescible organic wastes and particularly domestic sewage. In their day, the use of the term "biological indicators of pollution" when referring to organisms which respond in a certain way to heavy organic enrichment of their medium was perhaps justifiable. Untreated or inadequately treated domestic sewage then was by far the most important and perhaps the only well known and generally recognized water pollutant. Its discharge into public waters in amounts sufficient to bring about appreciable biotic changes being usually a hazard to human health, it was and is almost always pollution in any ordinary sense of the word. Today, the importance of pollutants other than domestic sewage is generally recognized. Yet, many authors still speak of "pollution indicators" when they actually are referring only to indicators of organic enrichment of water with putrescible organic wastes, which may or may not involve demonstrable damage to natural resources. Some readers are known to have been misled by this terminology, believing that the same biological indices are useful in detecting every kind of pollution.

Gaufin and Tarzwell (13), when reporting their studies of stream pollution with domestic sewage, obviously were considering the effects on aquatic life of an oxygen-depleting organic waste only. Nevertheless, such unqualified and seemingly general statements as their conclusion that "Pollutional associations are characterized by few species but large numbers of individuals" can be misleading. As the quoted authors well know, the numbers of many organisms initially present are reduced and the numbers of none are markedly increased in some waters polluted with toxic wastes, suspended solids such as silt, or even oxygen-depleting organic wastes discharged intermittently. These authors undoubtedly did not intend the conclusion in question to be a very broad generalization from their observational results having to do with one kind of pollution only. Their use of the expression "pollutional associations" for designating associations found in waters polluted with domestic sewage, or in waters enriched with putrescible organic matter, can be excused on the ground that no term that is more appropriate than the term "pollutional" has come into general use in the biological literature. Yet, this lack of a more precise terminology is not any less deplorable because the use of inappropriate terms, and terms which are not sufficiently specific, has become prevalent.

Beck (1) (2) explicitly confines his discussion to the subject of "organic pollution". He has proposed the use of a numerical "biotic index", which is said to be "indicative of the cleanliness (with regard to organic pollution) of a portion of a stream or lake" (2). He recognizes that his methods are "confined to fresh waters and encroaching salinity has a marked effect on the fauna of a stream." Inasmuch as many different pollutants, including toxic constituents of some organic wastes, likewise can have a marked effect on the fauna of a stream, it is apparent that Beck's methods may have only very limited applicability. It may be usable only in connection with the investigation and description of waters known in advance to contain no pollutants other than non-toxic putrescible organic matter.

Patrick (26) (27) (28), recognizing the importance of a variety of pollutants, apparently has attempted to devise a general procedure for the reliable biological detection and measurement of the different kinds of pollution. For reasons already indicated, however, this desirable objective appears to be attainable only when one defines pollution as "any thing which brings about a reduction in the diversity of aquatic life", which is not a generally acceptable definition.

Wurtz (38), while evidently realizing the existence and importance of a large variety of pollutants, seems to overlook completely the important differences of biotic responses to the different pollutants. Thus, his Figure 1 suggests that the same pollutional zones, including a "degradation zone" extending from the point of mixing of an effluent with the water of a stream to a "polluted zone" located some distance downstream, can be expected to occur in any heavily polluted stream, regardless of the nature of the pollutant (i.e., whether it be "organic", "toxic", or "physical"). Furthermore, he speaks of "pollution tolerant species" and of "non-tolerant organisms", suggesting that organisms are consistently tolerant or consistently non-tolerant with respect to all pollutants. Nowhere does he specify that he has in mind resistance to putrescible organic pollutants only, and there is considerable evidence that he has in mind all pollutants. In large degree, Wurtz seems to have adopted methods similar to Patrick's, but one of his innovations seems to require the probably impossible classification of all or nearly all aquatic organisms as "tolerant" and "non-tolerant" to all kinds of pollution, including the various toxicants, etc. Unfortunately, Wurtz does not include in his paper a list of all organisms considered by him to be tolerant and all those thought to be non-tolerant.

There can be no doubt that some of the so-called "pollution-tolerant" organisms, which actually are simply forms known to thrive in waters markedly enriched with organic wastes, are less tolerant with respect to some other water pollutants than a number of the species known as "clean-water" forms. For example, a species of Physa, a genus of snails generally believed to be resistant to organic pollution (1) has been found to be extremely susceptible to dissolved copper. Certain fish (e.g., centrarchids), may fly nymphs, etc., thought to be more susceptible than Physa to the effects of organic pollution, proved much more resistant to copper. An aquatic environment in which "clean-water" organisms are predominant might possibly be more seriously polluted than one with decidedly "pollutional" biota. The biological terminology evidently needs revision, so that the word pollution would not be used synonymously with organic enrichment.

It appears that, in general, very broad significance of the various biological indices of water quality and the severity of pollution has been only assumed and not actually demonstrated. This is well exemplified by the following quotations from the summary of one of Patrick's papers (27): "On the premise that the balanced physiological activities of aquatic life in surface waters are essential for the maintenance of healthy water conditions, it may be assumed that the most direct measure of this biodynamic cycle will indicate the condition of the water." It will be noted that we have here an assumption based upon a rather nebulous premise. Most writers have failed to supply entirely satisfactory, clear definitions of terms used (e.g., "pollution", "health", etc.) to show precisely what it is that they believe they can detect or measure biologically. Others have failed to use defined terms in a manner entirely consistent with their own definitions. The need for demonstration of the validity of some of the most fundamental assumptions concerning the reliability of pollution indices designed for general application has not been satisfied. Some authors seem to be of the opinion that the proof is unnecessary. It must be admitted that investigations designed to provide such proof would be extremely complex and difficult, and it is not likely that the search for this proof would be very rewarding, for there can hardly be a simple, general solution for the problem of pollution detection and measurement. Like a panacea, a general test for all kinds of pollutional damage is something for which biologists and engineers alike probably would be wise not to seek.

The value of fish as indicators of environment conditions and the importance of fish population studies in connection with the estimation of the intensity of water pollution now can be considered. Doubtless there is much more published information on the environmental requirements of fish than on the requirements of species of any other group of aquatic organisms excepting perhaps a few invertebrate species of outstanding economic importance. The vast quantity of published data relating to the water quality requirements of fish is partly revealed by a few recently prepared compilations and summaries of some of this information (4) (5) (8) (9) (10) (11) (17) (33). The resistance of many fish species to extreme temperatures, to unusual concentrations of dissolved oxygen and other dissolved gases, to variations of water salinity, and to extremes of pH, their susceptibility to the harmful effects of a great variety of toxic substances and of suspended solids of importance as water pollutants, the influence of some of these environmental factors upon embryonic development, growth, and activity, and so forth, have all been studied intensively. There exists also a voluminous literature on the food of fishes, their life history and reproductive requirements, their habitat preferences, movements, avoidance of adverse environmental conditions, and so on.

While it is evident that more is known of the environmental requirements of many fish than is known of the requirements of most, if not all, of the other aquatic organisms often considered as indicators of environmental conditions, the use of fish as indicators has received considerably less attention than has the use of other major groups, plant and animal, microscopic and macroscopic. Fisheries workers recognize the difficulty of adequately sampling fish populations even in bodies of water of moderate size, and this, along with the mobility of fishes, has been advanced as a reason for the unsuitability of fish as indicators of environmental conditions. But, other aquatic groups are difficult to sample too, as Needham and Usinger (25) have demonstrated in the case of the invertebrate macrofauna of a riffle. The difficulty of sampling and the mobility of fishes may not be the chief reasons why fish have not been given more consideration as indicators. The taxonomic groups which have received the most attention no doubt have reflected to some extent the special interests of investigators who happened to be working in the field of water pollution. Fish being the usual economic and recreational yield of stream productivity, their study has obvious applied value and so has required no additional justification. Further, the status of a fish population may indicate suitable or unsuitable environmental conditions, but when knowledge of this population is the end or aim of an investigation, the population status is not regarded as an index of anything else. The value of fish as indicators of the suitability of water for uses other than fishing has not been clearly demonstrated. Whatever the reasons may be, the emphasis in most discussions of the "biological indices" has been on groups other than fish, even though very little is known of the environmental requirements of the species of many of these groups.

The value of knowledge of fish populations in connection with the classification of aquatic environments has not been entirely overlooked. Ricker (32) made important use of the brook trout (Salvelinus fontinalis) and the Centrarchidae and Esocidae as a basis for his ecological classification of certain Ontario streams. Fisheries workers frequently use such expressions as "trout waters" or "bass waters", thus conveniently classifying waters according to the fish species for which the waters are well suited. European workers have made more formal use of such a system of stream classification (34) (37). Brinley and Katzin (3) have classified waters and named various pollutional "zones" of streams in the Ohio River drainage basin according to the kinds of fish populations found therein. As has been done with other animals and plants, some species of fish have been classified as to their "saprobic" preferences by a few authors (22) (24) (19) (35). The basis for such classification of fish is highly questionably. Patrick (26) (27) includes fish among the groups considered in her "biological measure" of stream conditions. Doudoroff (7) and Gaufin and Tarzwell (14) have emphasized the need for thorough fish population studies in connection with water pollution investigations and the determination of the pollutional status of waters.

Studies of fish populations in variously polluted waters, which reveal varying susceptibility of different fish species to pollutional conditions in their natural habitats, have been reported by a number of investigators (3) (6) (11) (20). However, sufficiently intensive sampling of fish populations has not often been undertaken in connection with routine pollution surveys and investigations, the sampling of other aquatic life having been probably more often emphasized when the scope of the biological studies has had to be limited. Inasmuch as it is not often possible adequately to study all of the aquatic biota, including the fish, the practical value of information to be obtained by concentrating attention on fish populations must be carefully weighed against that of information to be derived from equally intensive study of some of the other aquatic organisms, and from comparatively superficial study of the entire biota.

The absence or extreme scarcity of some fish in a stream below the point of entry of a waste, and not above the point of entry, strongly suggests that the waste is somehow detrimental to these fish; if valuable food and game fish species are among those believed to be adversely affected, pollution is indicated. Neither the presence nor the absence of fish is a reliable indication of suitability or unsuitability of water for domestic, agricultural, and industrial uses and for recreational uses other than fishing. Nevertheless, because of the great economic and recreational value of many fish species, this information is essential to sound classification of waters according to their pollutional status.

The presence of fish does not necessarily show that their environment has been suitable for them for a very long time, nor that the species found can survive indefinitely and complete their life cycles under the existing environmental conditions. However, the presence of thriving populations of non-migratory species, including numerous representatives of different age classes whose growth rates have not been subnormal, is significant. It suggests strongly that pollution which is highly detrimental to these fishes and to migratory species whose habitat preferences, natural food, and water quality requirements are quite similar has not occurred recently. For example, the presence of numerous cottids in Northwestern salmon and trout streams which receive organic wastes is believed to indicate that dissolved oxygen concentrations have been adequate for some time and other environmental conditions probably have been suitable not only for the cottids, but also for migratory salmon and trout. There is now no sound reason for believing that the presence of any invertebrate form is a more reliable and appropriate biological indicator of the suitability of past environmental conditions for the migratory salmonids than is the presence of cottids.

The value of waters used for fishing, and of the fisheries which they support, bears no fixed, direct relation to the number of fish species to be found therein, just as it bears no such relation to the number of species of other organisms present. Some 35 species of fish were collected in the Midwestern warm-water stream studied by Katz and Gaufin (20). Because of the scarcity of valuable food and game fishes, this small, polluted stream is not regarded as a valuable fishing stream. On the other hand, many cool, pure streams which are highly valued as trout and salmon streams contain very few fish species other than the salmonids. Indeed, the invasion of valuable trout waters by other fish species not initially present is generally regarded as evidence of degradation of these waters, for the numbers of trout usually decline when it occurs. Such a change of the fish population can be a result of increasing temperatures and probably also of enrichment (18). Warm, eutrophic waters can support a great variety of fish and other organisms, but trout waters which are approaching this condition can hardly be regarded as "healthy".

Some of the above statements seem to contradict Patrick's (26) (27) conclusion, based on a study of the Conestoga River Basin of Pennsylvania, that "The results of this study indicate that under healthy conditions a great many species representing the various taxonomic groups should be present." It is necessary, therefore, to examine the evidence on which the latter conclusion is based. It appears that, in accordance with Patrick's conception of what a "healthy" stream should be like biologically, only those stations where a variety of organisms judged to be fairly normal or typical was actually found were classed as "healthy". It is not surprising, therefore, that all of the stations classed as "healthy" had indeed this large variety of organisms. Chemical, bacteriological, and other data were collected and considered in selecting and classifying the stations studied. It is clearly indicated, however, that the variety of organisms found (which is the proposed index or measure of stream "health") also was a major consideration. Different conclusions perhaps would have been reached had the initial classification of the stations been based entirely on other criteria of obvious practical import (such as the abundance, condition, and growth rates of valuable native game fish, etc.) and had a greater variety of natural, unpolluted streams been examined. It is noteworthy also that certain stations which evidently were not much affected by waste discharges but lacked the usual variety of organisms (e.g., Station No. 152, in a stream section evidently suited for stocking with trout) were classed as "atypical" stations by reason of certain observed peculiarities, such as low water temperatures, unusual bottom or shore conditions, etc. Other stations which had the expected variety of organisms were classified as "healthy" stations despite noted peculiarities such as marked organic enrichment, unusually high BOD, high CO_2 content, high bacterial content, or great turbidity of the water. Thus, it appears that the rating of the stations was somewhat arbitrary.

When the possibility of certain pollutional damage specifically to fisheries is under consideration, it should be remembered that fishes have varying ecological requirements and habits, differ in their resistance to variations of water quality, and are not all dependent upon all aquatic organisms, nor upon the same organisms, for their food. It has been shown that the growth of some fish species is promoted in certain waters affected by the discharge of organic waste (21), whereas the same waters apparently are rendered unsuitable for some other species (20). A reduction of the number of species of fish-food organisms, with a great increase of abundance of some of the remaining species, which occur often in streams receiving various wastes, doubtless can be harmless or beneficial for some fish species, although this reduction may be detrimental to others. If they are not otherwise adversely affected by environmental changes, those fishes which can well utilize the abundant food organisms will thrive, while others may disappear. Whether the total effect on fisheries will be favorable or unfavorable clearly will depend on the relative commercial and recreational value of those fish populations which are favored and those which are affected adversely. An intensive study of the entire aquatic biota cannot always reveal the extent of pollutional damage to fisheries, unless the relative value of the various forms present (for man, or as food for important fishes) is considered.

To evaluate the effect of environmental changes on fisheries it is necessary to know what fish species were originally present, how highly each is valued, and in what way and to what degree each important species has been affected by waste discharges. The relative abundance and condition of individuals of different species in the waters under investigation and in suitable "control" areas, the growth-rates of different age classes, the palatability of the flesh, and possible interference with normal migratory movements or with other reproductive activities must all be considered. Fish collections taken by carefully planned netting will yield much of this information. Commercial and sport catch records, showing the take per unit of fishing effort, and various field observations (e.g., of spawning areas utilized, etc.) also can be very helpful. Inasmuch as the presence of wastes and other pollutants is by no means the only factor which can directly influence fish populations, the cause of observed differences of fish populations must be determined. In this connection, studies of the food of important fish species and of the relative abundance of available food organisms in waters which are affected and those which are not affected by waste discharges may be essential. However, if detection and evaluation of pollutional damage to fisheries is the only or primary objective of a biological investigation, an enumeration of the species of organisms of all taxonomic groups, or of some single invertebrate group, cannot be deemed a direct approach to the problem at hand. Judged only by its practical utility, it may be a waste of time, effort, and money, which perhaps could be far better expended on more directly pertinent studies. Indeed, it is difficult to imagine pollutional interference with any use or combination of uses of water which could usually be accurately and most efficiently evaluated in such an indirect manner.

A study of the influence of large amounts of organic waste on the ecology of the Tuolumne River of California has recently been completed by Warren (unpublished data). During August and September of 1952, the daily mean discharge rates of this river at the city of Modesto ranged from 293 to 822 cubic feet per second. The daily mean discharge rates of domestic and cannery waste introduced into the Tuolumne at Modesto ranged from 0 to 22.3 cubic feet per second. The 5-day biochemical oxygen demand of samples of this waste ranged from 60 to 575 parts per million. Dissolved oxygen concentrations at stations below the point of waste discharge ranged from zero to supersaturation during this time.

The objective of this study was to determine some of the effects of organic waste discharges on the ecology of the Tuolumne during the different seasons of the year. Some thirty miles of the river were studied, of which only the lower ten were influenced by waste discharges. The phytoplankton, zooplankton, benthic fauna, and fish were studied along with the physical, chemical, and bacteriological conditions in this river. The fishery phase of the investigation represented a small part of the total effort.

The investigation of the Tuolumne River now being complete and its objective more or less realized, it is interesting to consider how well other objectives might have been satisfied by this same study, planned and conducted as it was. For instance, had the objective been to determine the influence of the organic waste specifically on the fisheries of the Tuolumne, could not much of the effort devoted to the bacteriological, phytoplankton, zooplankton, and benthic faunal investigations have been far better expended on a thorough study of the fisheries? One is forced to conclude that were the objective to determine the status of the fisheries, the fish should have received most of the attention. This does not mean that studies of the plankton and of the benthic fauna are not necessary phases of an investigation so oriented. They may be quite necessary, but they should be so planned that the time and effort devoted thereto would not be out of proportion to their contribution to thorough understanding of the status or condition of the valuable fish populations.

The benthic fauna present at stations on the Tuolumne River below the point of waste discharge had many of the recognized "pollutional" characteristics during late summer and early fall. By this time, many of the "clean-water" species present at these stations earlier in the summer, and persisting at stations above the waste outfall, had disappeared. A marked reduction in species numbers had taken place, and at least one species occurred in unusually great numbers. While the bottom fauna showed changes that in accordance with most biological index methods would be regarded as evidence of pollution, rather intensive seining during mid-September resulted in the collection of 10 species of fish at stations above the point of waste discharge and 12 species at stations within the first ten miles below this point. The variety of fish present had certainly not been greatly altered by the introduction of wastes, even though the bottom fauna had been markedly modified.

Collections of young bluegills (Lepomis macrochirus) made in September showed the O-year class to grow faster at stations below the point of waste introduction than at stations above this point. The size difference persisted in the 1-year class. The difference in the O-year - class growth rates could probably be attributed to the greater abundance of zooplankton at the downstream stations.

While the above data are interesting, they cannot be taken as evidence that pollution of the Tuolumne damaging to fisheries did not exist. Some evidence indicated interference with a portion of the upstream migration of adult chinook salmon (Oncorhynchus tshawytscha), though the downstream migrant young were presumably unaffected, being apparently absent from the Tuolumne by the time of critical summer river flows and waste discharges. Juvenile shad may perhaps have been affected also. Had the principal objective of the Tuolumne River investigation been an evaluation of damage to fisheries resources by pollution, the study could not have been deemed complete in the absence of conclusive evidence that interference with salmon migrations and other possible damage to valuable fish populations had or had not occurred. None of the proposed "biological measures" of pollution intensity could have revealed the degree of such interference or damage. In order to obtain the crucial evidence required, it would have been necessary to emphasize the fisheries phase of the investigation.

It is not the purpose of this paper to discourage limnological research pertinent to water pollution problems, nor is it intended to deny the value of all biological indicators of pollution. There can be no doubt that a drastic modification of any natural aquatic biota, attributable to a change of water quality, can have highly undesirable aspects or consequences. Such changes presumably are detrimental to human use and enjoyment of natural waters more often than they are not. Many a readily demonstrable effect of wastes upon aquatic life in a valuable stream is suggestive of probable existing or incipient pollution which deserves close attention and investigation. Even before valuable fish populations have been materially affected by some potentially harmful pollutant, an observed detrimental effect upon other organisms which are somewhat more susceptible than fish may give warning of possible future damage to fisheries by continued or additional waste discharges. The nature and the source of existing or incipient pollution also may be revealed by appropriate biological indices. Finally, inasmuch as some of the organisms considered to be indicators of pollution are organisms which can directly interfere with human use or enjoyment of waters (e.g., unsightly slime-forming organisms such as Sphaerotilus, odor-producing algae, etc.), their unusual abundance may not be disregarded in evaluating over-all damage caused by pollution.

CONCLUSIONS

It must be concluded that every change or peculiarity of the flora and fauna of a stream which has been referred to as an index or measure of pollution is in reality only an index of environmental disturbance or environmental anomaly. The disturbance or anomaly indicated may or may not be pollutional in the sense that stream uses are interfered with. Pollution (i.e., interference with stream uses) can be negligible when the effect on the aquatic biota as a whole is great, and it can be severe when most of the aquatic life is unaffected. Gross pollution often can be demonstrated without any biological investigation. When biological investigation may be necessary, pollutional damage to valuable aquatic organisms can probably best be determined by concentrating attention upon these particular organisms. Yet, since all aquatic life forms are more or less sensitive to changes of water quality, the fate of any of them theoretically can be instructive, revealing something about the nature and magnitude of these changes that may not be obvious nor easily determined otherwise.

A genuine contribution to water pollution science can be made whenever the presence or relative abundance of living organisms of any kind can be shown to be a reliable index of something tangible that one may need to know in order fully to ascertain and understand the pollutional status of an aquatic environment. When proposing and describing the use of such biological indices, one should state specifically what it is that each is believed to indicate, carefully avoiding such general, vague, or abstract terms as "pollution" and "stream health", which may be variously understood. Does it indicate, for example, continual presence of dissolved oxygen in certain concentrations believed to be adequate for sensitive fish species? Does it indicate organic enrichment likely to interfere in some way other than through oxygen depletion with certain specific uses of water? Or does it indicate that particular toxic substances have not recently been present in concentrations likely to be injurious to fish, to man, or to certain crops? No simple biological indicator and no one measure of stream conditions can indicate all of these things. But any species can become a biological indicator of environmental conditions of possible interest as soon as its nutritional and other environmental requirements, its relative resistance to various toxic substances, etc., become known. Widely distributed sessile or sedentary organisms should be the most useful indicators of past conditions. Unfortunately, the water quality requirements of most of the "indicator organisms" have never been thoroughly investigated, so that there is no real knowledge of specific factors which limit their distribution and abundance. Probably nobody now knows just why any of the so-called clean-water organisms begin to disappear from waters subject to progressively increasing organic enrichment. Here is a field for future research which is far more promising than is, for example, the questionable classification of all aquatic organisms as "pollutional", "clean-water", or "facultative". If there are common sedentary organisms whose water quality requirements can be shown to correspond closely with those of valuable fish species, they are potentially useful indicators. At the present time, however, excepting instances of gross pollution, only fish themselves can be said to indicate reliably environmental conditions generally suitable or unsuitable for their existence.

REFERENCES

1. Beck, W.M. 1954. Studies in stream pollution biology. I. A simplified ecological classification of organisms. Quarterly journal of the Florida Academy of Science, 17:211-227.

2. Beck, W.M. 1955. Suggested method for reporting biotic data. Sewage and Industrial Wastes, 27: 1193-1197.

3. Brinley, F.J. and L.I. Katzin. 1944. Biological studies. Ohio River Pollution Control. Supplements to Part 2, Report of the U.S. Public Health Service. 78th Congress, 1st Session, House Document No. 266, pp. 1275-1368.

4. California State Water Pollution Control Board. 1952. Water quality criteria. California State Water Pollution Control Board, Publication No. 3. Sacramento. (See also Addendum No. 1, 1954)

5. Cole, A.E. 1941. The effects of pollutional wastes on fish life. In A Symposium on Hydrobiology, University of Wisconsin Press, Madison, pp. 241-259.

6. Dimick, R.E. and F. Merryfield. 1945. The fishes of the Willamette River system in relation to pollution. Oregon State College Engineering Experiment Station Bulletin Series, No. 20.

7. Doudoroff, P. 1951. Biological observations and toxicity bioassays in the control of industrial waste disposal. Proceedings of the Sixth Industrial Waste Conference, Purdue University Engineering Extension Bulletin, Series No. 76, pp. 88-104.

8. Doudoroff, P. In Press. Water quality requirements of fishes and effects of toxic substances. In The Physiology of Fishes (M.E. Brown, Editor). The Academic Press, New York.

9. Doudoroff, P. and M. Katz. 1950. Critical review of literature on the toxicity of industrial wastes and

their components to fish. I. Alkalies, acids, and inorganic gases. Sewage and Industrial Wastes, 22:1432-1458.

10. Doudoroff, P. and M. Katz. 1953. Critical review of literature on the toxicity of industrial wastes and their components to fish. II. The metals, as salts. Sewage and Industrial Wastes, 25:802-839.

11. Ellis, M.M. 1937. Detection and measurement of stream pollution. Bulletin of the Bureau of Fisheries, 48:365-437.

12. Fjerdingstad, E. 1950. The microflora of the river Molleaa with special reference to the relation of the benthal algae to pollution. Folia Limnologica Scandinavica No. 5.

13. Gaufin, A.R. and C.M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Public Health Reports, 67:57-64.

14. Gaufin, A.R. and C.M. Tarzwell. 1953. Discussion of R. Patrick's paper on "Aquatic organisms as an aid in solving waste disposal problems." Sewage and Industrial Wastes, 25:214-217.

15. Gaufin, A.R. and C.M. Tarzwell. 1955. Environmental changes in a polluted stream during winter. The American Midland Naturalist, 54:78-88.

16. Gaufin, A.R. and C.M. Tarzwell. 1956. Aquatic macro-invertebrate communities as indicators of organic pollution in Lytle Creek. Sewage and Industrial Wastes, 28:906-924.

17. Harnisch, O. 1951. Hydrophysiologie der Tiere. Schweizerbart'sche, Stuttgart. (Die Binnengewässer, Vol. 19)

18. Hasler, A.D. 1947. Eutrophication of lakes by domestic drainage. Ecology, 28:383-395.

19. Johnson, J.W.H. 1914. A contribution to the biology of sewage disposal. Journal of Economic Biology, 9:117-121.

20. Katz, M. and A.R. Gaufin. 1953. The effects of sewage pollution on the fish population of a Midwestern stream. Transactions of the American Fisheries Society, 82:156-165.

21. Katz, M. and W.C. Howard. 1955. The length and growth of O-year class creek chubs in relation to domestic pollution. Transactions of the American Fisheries Society, 84:228-238.

22. Kolkwitz, R. 1911. Biologie des Trinkwassers, Abwassers und der Vorfluter, Rubner, Gruber und Ficker's Handbuch des Hygiene. II. S. Hirzel, Leipzig.

23. Kolkwitz, R. and M. Marsson. 1908. Oekologie der pflanzlichen Saprobien, Berichte der Deutschen Botanischen Gesellschaft, 26a:505-519.

24. Kolkwitz, R. and M. Marsson. 1909. Oekologie der tierischen Saprobien. Internationale Revue der gesamten Hydrobiologie und Hydrographie, 2:126-152.

25. Needham, P.R. and R.L. Usinger. 1956. Variability in the macrofauna of a single Riffle in Prosser Creek, California, as indicated by the Surber sampler. Hilgardia, 24:383-409.

26. Patrick, R. 1949. A proposed biological measure of stream conditions, based on a survey of the Conestoga Basin, Lancaster County, Pennsylvania. Proceedings of the Academy of Natural Sciences of Philadelphia, 101:277-341.

27. Patrick, R. 1950. Biological measure of stream conditions. Sewage and Industrial Wastes, 22:926-938.

28. Patrick, R. 1953. Biological phases of stream pollution. Proceedings of the Pennsylvania Academy of Science, 27:33-36.

29. Patrick, R. 1953. Aquatic organisms as an aid in solving waste disposal problems. Sewage and Industrial Wastes, 25:210-214.

30. Patrick, R., M.H. Hohn, and J.H. Wallace. 1954. A new method for determining the pattern of the diatom flora. Notulae Naturae, No. 259.

31. Richardson, R.E. 1928. The bottom fauna of the Middle Illinois River, 1913-1925. Illinois Natural History Survey Bulletin, 17:387-475.

32. Ricker, W.E. 1934. An ecological classification of certain Ontario streams. University of Toronto Studies, Biological Series No. 37.

33. Southgate, B.A. 1948. Treatment and disposal of industrial waste water. Department of Scientific and Industrial Research (Gr. Brit.), London.

34. Steinmann, P. 1915. Praktikum der Susswasserbiologie. Verlag Borntraeger, Berlin.

35. Suter, R. and E. Moore. 1922. Stream pollution studies. Bulletin, New York State Conservation Commission.

36. Tarzwell, C.M. and A.R. Gaufin. 1953. Some important biological effects of pollution often disregarded in stream surveys. Proceedings of the Eighth Industrial Waste Conference. Purdue University Engineering Extension Bulletin, Series No. 83, p. 295-316.

37. Thienemann, A. 1925. Die Binnengewässer Mitteleuropas. Schweizerbart'sche, Stuttgart. (Die Binnengewässer, Vol. 1)

38. Wurtz, C.B. 1955. Stream biota and stream pollution. Sewage and Industrial Wastes, 27:1270-1278.

Reproduced With Permission From:
ECOLOGY
39(1958): 530-535

BIOACCUMULATION OF RADIOISOTOPES THROUGH AQUATIC FOOD CHAINS*

J. J. Davis and R. F. Foster
Hanford Laboratories Operation, General Electric Company, Richland, Washington

INTRODUCTION

With an increasing number of atomic energy installations and their associated problems of disposal of liquid wastes, we recognize that more and more aquatic environments are going to be exposed to at least low concentrations of radioactive materials. For the safety of human populations who may be drinking water which contains such radioactive materials, a set of maximum permissible concentrations has been recommended (International Commission on Radiological Protection, 1955). By themselves, however, such recommendations are inadequate to define completely the radiological hazard which may develop through aquatic food chains. Where biological systems are involved, the organisms may accumulate certain isotopes to many times the initial concentrations in the water. There are many radioisotopes, however, that apparently are not biologically concentrated.

This paper describes some of the mechanisms involved in the accumulation of radioisotopes by aquatic organisms, with special reference to food webs and metabolic rates, and presents some examples of how the concentration of radioisotopes in organisms can be used to measure relationships between different species.

THE ACCUMULATION OF RADIOACTIVE MATERIALS

In order to interpret the reasons for, or to predict the concentration of, radioactive substances in aquatic forms, the biologist must appreciate that several basic processes are involved. The most important are: (1) the mode of uptake, which includes adsorption to exposed areas, absorption into tissues, and assimilation of ingested material; (2) retention, which is a function of the biochemistry of the particular elements and components involved, the site of deposition, the turnover rate, and the radioactive half-life; and (3) the mode of elimination, which may involve ion exchange, diffusion, excretion, and defecation.

MODE OF UPTAKE

The metabolism of the different radioelements and the relative importance of the different modes of uptake will fluctuate widely between different species, environments, and seasons. While this paper is principally concerned with assimilation through food chains, the processes of adsorption and absorption of radioactive substances directly from the water cannot be neglected. They are primary mechanisms by which inorganic materials are acquired by aquatic plants which are the food sources of the animals. The absorption of radioisotopes of strontium, barium-lanthanum and sodium by fresh-water fish has been demonstrated by Prosser et al. (1945). Absorption of radio-calcium has been demonstrated by Lovelace and Podoliak (1952) and by Rosenthal (1956). Chipman (1956) showed that cesium readily passed through excised pieces of tuna skin but that there was little absorption of strontium or ruthenium from sea water. Fish immersed in effluent from the Hanford reactors concentrated Na^{24} in the tissues about 130-fold. Direct absorption of other isotopes which are dominant in the effluent, including Cr^{51}, Cu^{64}, P^{32}, As^{76}, and rare earths, appeared to be inconsequential, however. In fish that live downriver from the Hanford reactors, sorption of radioactive materials directly from the effluents accounts for only about 1.5 percent of the total radioactivity. Consequently, sorption is of much less importance than ingestion in the uptake of radioactive materials by Columbia River fish.

Adsorption occurs almost instantaneously, as has been demonstrated with yttrium on cells of the marine alga Carteria by Rice (1956), while equilibrium by absorption is usually reached by algal cells (Whittaker, 1953) and by vascular aquatic plants (Hayes, et al., 1952) within a few hours. Because of the rapid uptake of radioisotopes by these mechanisms, Columbia River plankton, composed almost entirely of diatoms, appears to reach equilibrium about one hour after floating into the zone containing effluent from the Hanford reactors (Foster and Davis, 1955).

Assimilation of ingested materials is the dominant means by which many radioactive materials become accumulated in animals since the bulk of their essential elements is obtained from their food. The contribution of food webs to the concentration of radioisotopes in aquatic animals was apparent from samples collected from the Columbia River soon after the first Hanford reactors began operation. Fish collected downriver from the reactors were approximately 100 times as radioactive as laboratory fish that were exposed to equivalent mixtures of the effluent, but fed uncontaminated food. Bottom animals, particularly herbivorous insect larvae, were found to be even more radioactive than the fish. The concentrations of radioactive materials in Columbia River organisms have never approached hazardous levels, however.

DIFFERENCES BETWEEN SPECIES

The relative concentrations of beta emitters in various Columbia River organisms are shown in Figure 1. There are several reasons for the differences

---

* Presented at the Symposium on Radio-Ecology at the meeting of the American Institute of Biological Sciences, University of Connecticut, Storrs, Connecticut, August 27-30, 1956.

This paper is based on work performed under contract No. W-31-109 Eng-52 for the U.S. Atomic Energy Commission. The authors wish to express their gratitude to Dr. H.A. Kornberg for stimulating interest in the mathematical expression of some of the basic concepts, and for his general guidance of the biological studies at Hanford which have furnished most of the examples used here. We are also grateful to members of the Aquatic Biology Operation, particularly Mr. P.A. Olson and Mr. D.G. Watson, who contributed data which has not been published elsewhere.

which occur between these species.

(1) Several radioisotopes are involved and their relative proportions are different in the various organisms. A good indication of the proportions of the several isotopes can be obtained from curves like those in Figure 2 which show the characteristics of the radioactive decay of the isotope mixtures peculiar to each species. The positions of the curves in Figure 2 at zero time approximate the relative concentrations of radioisotopes in the water, small fish [*Richardsonius balteatus* (Richardson)], caddis larvae [*Hydropsyche cockerelli*, Banks], and plankton of the Columbia River during late summer months. The predominance of short-lived isotopes in the water is shown by the steep slope of the bottom curve. Short-lived emitters also contribute most of the radioactivity in the plankton but these have virtually disappeared by the fifth day. The remaining activity in the plankton, which is only about 20 percent of that originally present, emanates from P^{32} and other isotopes with half-lives greater than two weeks. In the caddis larvae and fish, only about 5 percent of the initial radioactivity originates from short-lived emitters. After the first day the rate of decay is quite uniform and characteristic of P^{32} (half-life 14.3 days). The dominance of the P^{32} has been confirmed by radiochemical analysis.

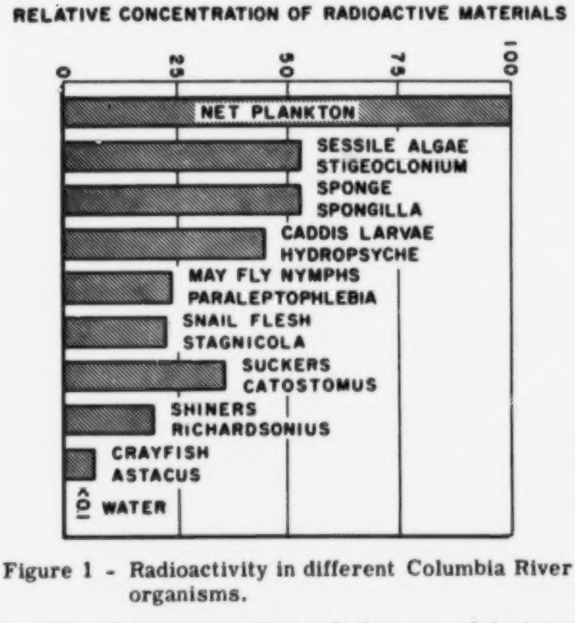


Figure 1 - Radioactivity in different Columbia River organisms.

The relative proportions of the several isotopes differ from one organism to another not only because of dissimilarities in the chemical composition and physiological demands of the different forms but also because of the different sorption characteristics which vary with morphology. Food chains are also important since they tend to "select for" isotopes of the essential elements, in this case P^{32}, and to "select against" nonessential elements. During the late summer months, the concentration of P^{32} in small fish of the Columbia River may be 165,000 time that of the water. On the other hand, As^{76} is barely detectable in the fish although it is responsible for a substantial fraction of the radioactivity in the water.

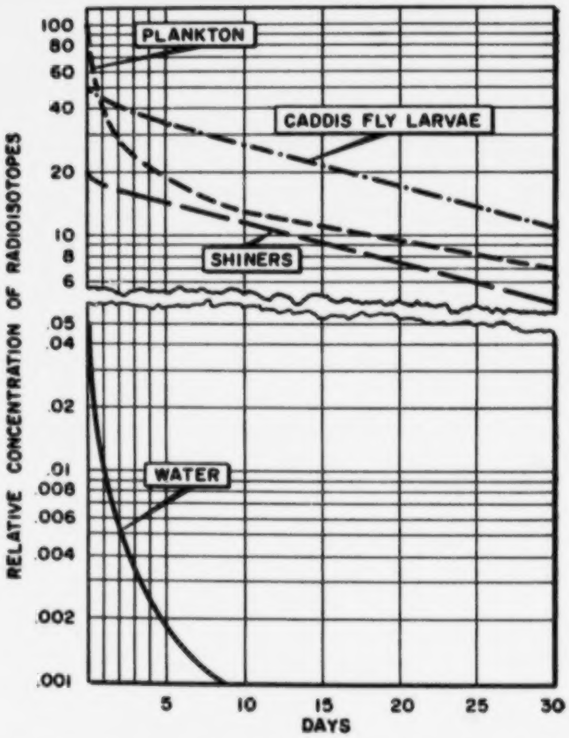


Figure 2 - Radioactive decay in different organisms and Columbia River water.

The marked variation in the relative abundance of different isotopes which can occur at different trophic levels and even between similar species has recently been pointed out by Krumholz (1956). From data collected at White Oak Lake, which received a variety of radioactive wastes from the Oak Ridge National Laboratory, Krumholz states: "Although radiophosphorus was generally accumulated in much greater amounts than any other radioelement by the organisms that served as food for the fish, that element made up only a small portion of the total radiomaterials concentrated in the fish tissues; whereas radiostrontium, which was present in the food organisms in only relatively small quantities, was accumulated in high concentrations in the fish skeletons. Furthermore, although the contents of the bluegill stomachs contained more radioactivity, on the average, than those of the black crappies, the crappies accumulated considerably greater amounts of radiomaterials in the hard tissues than the bluegills did. The bluegills, on the other hand, accumulated more radiomaterials in the soft tissues than the black crappies. Both species concentrated radiostrontium in quantities 20,000 to 30,000 times as great as those in the water in which they lived."

(2) Variation in moisture content between different organisms is a second reason for the differences in concentration of radioisotopes shown in Figure 1. Chemical composition is also a factor since we are

actually concerned with the quantity of a particular element in a unit mass of live tissue. The percentage of the live weight of the Columbia River plankton, caddis larvae, and minnows which is contributed by the inorganic ash is respectively 16, 2.2, and 3.0; and the concentration of phosphorus in the living organisms is about 150 ppm for plankton, 2,000 ppm for caddis larvae, and 6,000 ppm for minnows. Even greater differences may occur between the different tissues of an individual. Figure 3 shows how the concentration of radioactive materials varies between different tissues of whitefish in the Columbia River. Since virtually all of the activity is from P^{32} this gives a good indication of the relative concentration of phosphate in the different tissues.

(3) A third reason for differences in concentrations of radioisotopes between different organisms is their relative position on the food pyramid. Although elements are exchanged continuously between the water and the organisms of a food web, there is a mean retention time for each element in each organism. Each trophic level thus serves as a kind of pool or reservoir in which essential elements are retained for some mean length of time before they are passed on to the next level. The size of each pool will be governed by the total amount of an element held by the entire biotic mass making up the particular trophic level. A major fraction of most radioactive contaminants accumulated by aquatic life will be held by the plankton and benthic algae because of their relatively large total mass. Rigler (1956) found that over 95 percent of the P^{32} added to a lake was taken up by plankton (including bacteria) within 20 minutes. But retention time is not necessarily a function of the size of the pool. Indeed it is more apt to be inversely related since most elements will remain for a longer time in the larger organisms than in the small plant forms, although the small plants constitute the largest pool. Since, in the Columbia River, we are dealing with a flowing stream where isotopes are added at a more or less constant rate, much of the mineral exchange system can be considered as a once-through process rather than a cycle. Some radioactive decay will occur while the isotopes are retained in each trophic level. This decay, and thus the effective retention period, should be measurable by a progressive decline in specific activity -- the concentration of an isotope per unit mass of the element. For example, under certain conditions midge larvae in the Columbia River may contain on the order of 4 μc P^{32}/g of P and the small fish which eat the midge larvae about 0.5 μc P^{32}/g of P . Since the half-life of P^{32} is two weeks, the phophorus deposited in the fish must be, on the average, about six weeks "older" than that in the midge larvae. The relative "age" of the isotope will differ between species and differ between species and will change with the age, sixe, and growth rate of the individual and with the seasons. The decrease in specific activity will, of course, be more apparent for short-lived isotopes than for those with half-lives of several weeks or more.

The specific activity of the river biota should be appreciably lower than that of the water not merely because of the time required to incorporate the isotope into the organisms but also because of the "pools" of elements fixed in the biota and sediments. When a radioisotope is first introduced into a body of water it will be isotopically diluted with the stable form of the element which is dissolved in the water. Soon, it also will become isotopically diluted by exchange with the stable form of the element which has not been in solution. With a single addition of isotope into a "static" environment, the specific activity will eventually become uniform throughout the biota. Reservoirs of phosphates in the solids of lakes have been described by Hutchinson and Bowen (1950) and Hayes and coworkers (1952), who have studied phosphorus exchange with the use of P^{32} .

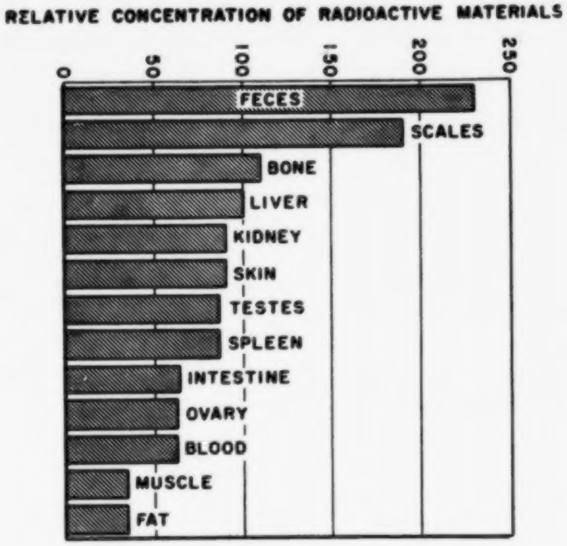


Figure 3 - Radioactivity in different tissues of Columbia River fish.

RATE OF ACCUMULATION BY AQUATIC ORGANISMS

The nearly instantaneous uptake of isotopes by adsorption and the rapid uptake by absorption have been mentioned. When animals are chronically feeding on radioactive materials, the rate at which their concentration of the isotopes approaches equilibrium will be a function of the radioactive and biological half-lives of the particular isotope involved.

Figure 4 shows the rate at which caddis fly larvae (*Hydropsyche cockerelli*) accumulated radioactive materials (mostly P^{32}) when fed filamentous algae (mostly *Spirogyra*) that had been cultured in reactor effluent. If there was no biological turnover of phosphorus in the caddis larvae, the time required to reach some fraction of the equilibrium level would be a function of the radioactive decay constant and could be predicted from the equation:

$$\frac{Q_t}{Q_e} = 1 - e^{-\lambda t}$$

where Q_e is the amount of the isotope present at equilibrium, Q_t is the amount present at some time (t) before equilibrium is reached, and λ is the radioactive decay constant.

Since true equilibrium will only be reached after infinite time, we can consider practical equilibrium to occur when $Q_t = 0.9\,Q_e$, and solve the equation for t. For any isotope, t will be equal to the half-life multiplied by $\frac{-\ln 0.1}{.693}$. For P^{32} it is approximately 47 days.

The curve presented in Figure 4 shows a much shorter time which indicates that significant biological turnover is present. The equation is easily modified to take this into account:

$$\frac{Q_t}{Q_e} = 1 - e^{-\delta t}$$

where δ is the sum of λ and β, where β is the constant for biological half-life. A 0.9 of equilibrium,

$$t = \frac{-\ln 0.1}{\beta + \lambda}$$

From Figure 4, t is about 50 hours and

$$50 = \frac{2.302}{\frac{.693}{T_b} + \frac{.693}{343}}$$

(343 is the half-life of P^{32} in hours).

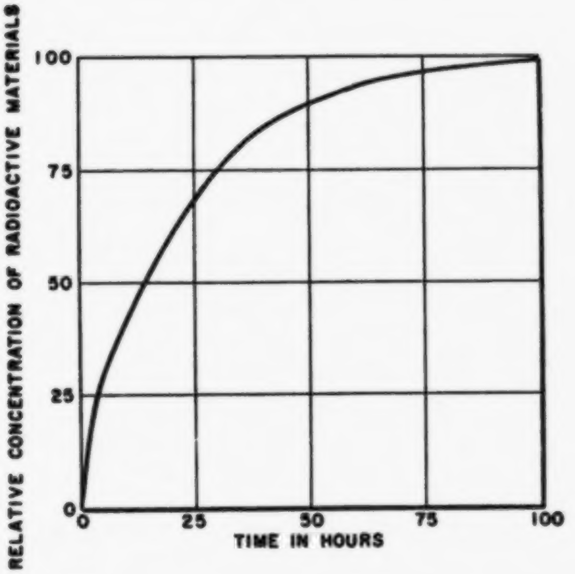


Figure 4 - Rate of accumulation of effluent isotopes by caddis fly larvae.

The biological half-life, T_b , is about 16 hours. Under such conditions the specific activity of the P^{32} will not diminish appreciably at this trophic level.

If laboratory tests can be carried out in conjunction with field observations, some interesting ecological relationships can be deduced. For example, we might measure the concentration of P^{32} in small minnows collected from a contaminated environment and find this to be 2×10^{-3} μc/gram. If the average size of the minnows was 5 grams, then each fish would have a body burden of about 10^{-2} μc of P^{32}. From a laboratory test, which duplicated field conditions as closely as possible, we might find that 0.9 of Q_e was reached in 20 days. Then

$$\delta = \frac{-\ln 0.1}{t} = \frac{2.302}{20} = 0.115$$

The same test might show that half of the ingested P^{32} was assimilated and deposited ($a = 0.5$). Assuming the concentration of P^{32} in the river fish to be in equilibrium with the environment and neglecting growth.

$$Q_e = \frac{aq}{\delta}$$

where q is the quantity of P^{32} ingested per unit time -- in this case each day. Then,

$$10^{-2}\,\mu c = \frac{0.5\,q}{0.115}$$

$$q = 2.3 \times 10^{-3}\,\mu c/\text{day}.$$

and

In order to have reached the observed concentration of P^{32}, each minnow must have consumed about 2.3×10^{-3} μc of P^{32} each day. If, from stomach analyses, we have found that the fish feed predominantly on midge larvae and from field collections we have found that the midge larvae have a concentration of P^{32} of about 10^{-2} μc/g , then we can surmise that each minnow has been eating about 0.23 grams of the midge larvae each day.

SEASONAL VARIATIONS

Since most aquatic animals are poikilothermic, their metabolic rates, and thus their feeding rates, change with variations in temperature and so with the seasons. For those aquatic forms that accumulate radioactive substances principally via ingestion, the concentration of radioisotopes fluctuates with metabolic rate. Figure 5 shows the seasonal fluctuations which occur in the radioactivity of plankton (diatoms) and minnows (Richardsonius balteatus) in the Columbia River. Fluctuations in plankton are quite similar to those in the water since the radioisotopes are acquired by direct absorption and adsorption (Foster and Davis, 1955). On the other hand, fluctuation in the radioactivity of the minnows is more closely related to the temperature. The 75-fold increase in concentration of radioisotopes in the fish between winter and late summer does not mean simply that the fish are eating 75 times as much food. The seasonal fluctuations result from the interaction of all of the factors mentioned above which influence the accumulation of radioactive materials. As the feeding rate increases for each organism, its intake of radioisotopes may be disproportionately large. The consumer is not only eating more grams of food, but each food organism has become more radioactive, and the effective time intervals between trophic levels have become less. Possibly the food habits of the species in question have also changed. A complete evaluation of the seasonal fluctuations in any one species would require an immense amount of work, not only on the food habits of the species but also on its physiology and on the radioactive contamination of its food organisms.

Not all seasonal variations are associated merely with temperature since deviations may occur where complex life cycles are involved. This occurs in immature insects which are less radioactive during quiescent periods than when the larvae or nymphs are feeding. It is also true of salmon that return to the Columbia River to spawn. The adult salmon virtually stop feeding when they enter fresh water, and consequently pick up very little radioactive material. Krumholz (1956) also observed definite seasonal changes in the accumulation of radiomaterials by fish of White Oak Lake. These corresponded to some extent with seasonal changes in temperature. He noted, however, that the accumulation of radioisotopes in black crappie and bluegills stopped at the first of August when the temperature reached about 80° F. He attributes the rapid loss of radioactive materials during August and September to a period of summer dormancy for these species.

SUMMARY

Some radioactive materials introduced into aquatic environments may be accumulated by the organisms. The amount of accumulation will vary over many orders of magnitude depending upon the kinds of isotopes involved and many physical, chemical, and biological factors. Such concentration is of considerable importance in the control of radiological hazards and the aquatic biologist has definite responsibilities in this area.

The processes of adsorption and absorption are of major importance in the uptake of radioisotopes by plants but appear to be of less importance than the food chain in the uptake by aquatic animals. The concentration of radioactive substances will vary between species and tissues and will fluctuate according to food habits, life cycles, and seasonal changes.

Within the biotic mass, a major fraction of most radioactive contaminants will be held by the organisms which make up the primary trophic levels. In a flowing stream, the specific activity of a radioisotope will diminish along the food chain. Where the turnover rates of certain isotopes can be measured, inferences can be drawn on feeding habits.

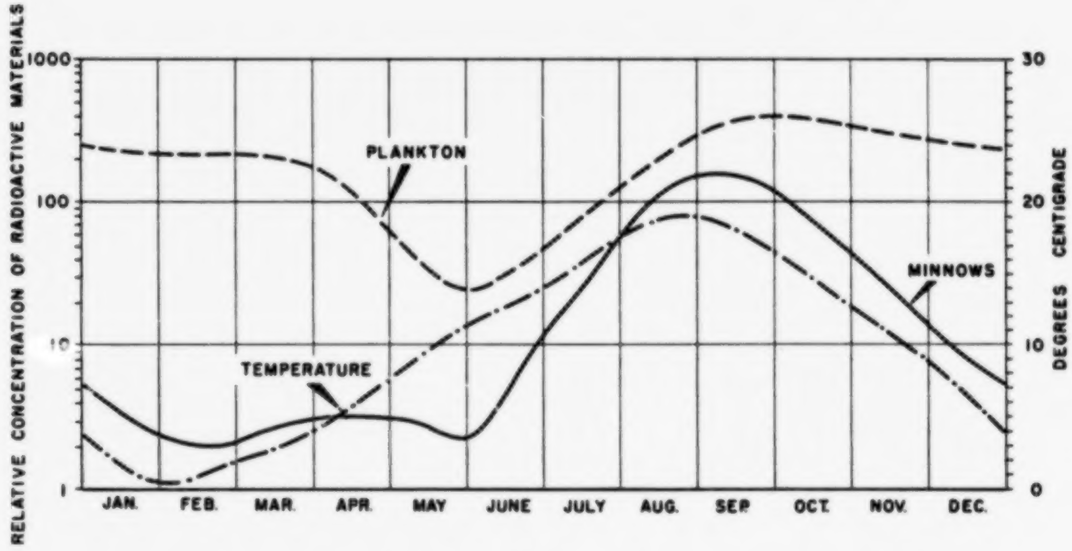


Figure 5 - Seasonal fluctuations in radioactivity of Columbia River organisms.

REFERENCES

Chipman, Walter A. 1956. Passage of fission products through the skin of tuna. U.S. Fish and Wildlife Service Special Scientific Report - Fisheries No. 167.

Foster, R.F. and J.J. Davis. 1955. The accumulation of radioactive substances in aquatic forms. Proceedings of the International Conference on the Peaceful Uses of Atomic Energy, 13 (P/280) : 364-367.

Hayes, F.R., J.A. McCarter, M.L. Cameron, and D.A. Livingstone. 1952. On the kinetics of phosphorus exchange in lakes. Jour. Ecol. 40 : 202-216.

Hutchinson, G.E. and V.T. Bowen. 1950. Limnological studies in Connecticut. IX. A quantitative radiochemical study of the phosphorus cycle in Linsley Pond. Ecology 31 : 194-203.

International Commission. 1955. Recommendations of the international commission on radiological protection. British Jour. of Radiology, Suppl. No. 6, 92 pp.

Krumholz, L.A. 1956. Observations on the fish population of a lake contaminated by radioactive wastes. Bull. Am. Mus. Nat. Hist. 110 : 277-368.

Lovelace, F.E. and H.H. Podoliak. 1952. Absorption of radioactive calcium by brook trout. The Progressive Fish-Culturist 14 : 154-158.

Prosser, C.L., W. Pervinsek, Jane Arnold, G. Svihla, and P.C. Thompkins. 1945. Accumulation and distribution of radioactive strontium, barium-lanthanum, fission mixture and sodium in goldfish. USAEC Document MDDC-496 : 1-39.

Rice, T.R. 1956. The accumulation and exchange of strontium by marine planktonic algae. Limnology and Oceanography 1 : 123-138.

Rigler, F.H. 1956. A tracer study of the phosphorus cycle in lake water. Ecology 37 : 550-562.

Rosenthal, H.L. 1956. Uptake and turnover of calcium-45 by the guppy. Science 34 : 571-574.

Whittaker, R.H. 1953. Removal of Radiophosphorus contaminant from the water in an aquarium community. In Biology Research -- Annual Report 1952. USAEC Document HW-28636 : 14-19.

46.A

Chapter II

RELATIONSHIP TO POLLUTION OF PLANKTON

Reproduced With Permission From:
BERICHTE DER DEUTSCHEN BOTANISCHEN GESELLSCHAFT.
(REPORTS OF THE GERMAN BOTANICAL SOCIETY)
26a(1908) : 505-519
TRANSLATION BY UNITED STATES JOINT PUBLICATIONS RESEARCH SERVICE
WASHINGTON, D.C.

ECOLOGY OF PLANT SAPROBIA

R. Kolkwitz and M. Marrson

The present report contains a listing of approximately 300 plant organisms of importance for the self-purifying capacity of our native waters. In order to express their dependence on decomposing organic nutrients, we introduced in 1902 (1) the term "saprobia" for them and subdivided them into poly-, meso- and oligosaprobia in accordance with the increasing degree of mineralization in such waters.

Such a classification presupposes that the respective organisms are uniquely dependent, within relatively narrow limits, on the chemical composition of the water for their distribution and development in situ. The Institute mentioned has available a very large number of analyses of the composition of a great many different bodies and courses of water and many years of investigation have shown that this assumption is valid if we take into account in such a classification an ecology which assigns a higher value to the occurrence of floristic constituents -- insofar as these develop typically -- than the determination of merely isolated specimens. The present case therefore concerns a classification of aquatic plants on a chemo-physiological basis in which laboratory tests are less decisive than the findings in situ of their presence. Such locations are collectors or channels for raw putrescible sewage, filtered effluents from trickle fields or biological oxidation, settling or collecting tanks absorbing nutrient-containing inflow as well as overgrown ponds, cisterns and dry wells.

For reasons of lack of space, we have temporarily omitted from consideration the organisms of pure water (catharobia), specifically their plankton and also because the term saprobia is hardly applicable to them as in the case of a number of algae from pure mountain streams and lakes. Here we should point out that our present observations indicate that there are very few chlorophyll-containing organisms which refuse any organic nutriment under natural conditions and that there are hardly any surface waters which do not react to permanganate through their content of organic substances.

The already mentioned extensive and definitive influence of aquatic organisms, especially when microscopic, has already been utilized by Ferd. Cohn to a limited extent for the evaluation of the degree of purity of these waters as a function of the organisms existing in them. This method of biological analysis was therefore developed in the botanical field. With its further development must be credited Mez, Schorlein, Lindau, Schmidmann (by founding the Institute mentioned), Schiemenz and Hofer among many others.

The large-scale experiments most appropriate for testing the relation between organisms and the character of the water have steadily increased in Germany since 1870 by reason of the increased outflow from the sewers of the growing towns and cities and through the increased volume of sewage from industrial agricultural enterprises. Obviously, methods of purification have been perfected at the same time which have also lead to valuable and pertinent experimental findings. In spite of certain differences in chemical composition, all these effluents produce the same biological picture in general in the collectors because a stream or lake tends to compensate the existing differences by dilution, neutralization, oxidation, etc. and thus creates the same similar nutritive conditions for saprobia.

A detailed discussion of the considerations in classifying the organisms contained in the attached list into the respective zones will follow in the Reports of the Institute, together with a discussion of the respective animals and with the addition of illustrations. In this report here, we assume familiarity with all the organisms listed as well as the knowledge of their general habitat within the three typical regions of bank, bottom (benthos) and open water (plankton) and limit ourselves to giving a brief characteristic of the three different zones.

I - The zone of polysaprobia is characterized from the biological viewpoint principally through the wealth of schizomycetes by number, species and variety. Polysaprobia may gradually overlap into the zone of mesosaprobia (cf. Spherotilus) but will never occur grouped in the oligosaprobiotic zone but only in isolation and then generally erratically. The overlapping of Spherothilus into the second zone finds its reason in the fact that Spherotilus is an inhabitant of running water and needs aeration in addition to motion.

Designations such as "in pure and impure water" or "Euglena viridis is found in all Euglenaceae habitats" are offenses against the ecological viewpoint in our system. The number of germs capable of development per cubic centimeter of standard nutrient gelatine can easily rise above 1 million. Our standard food fishes can become easily subject to asphyxiation in this zone.

From the chemical viewpoint, the zone of the polysaprobia is characterized by the predominance of reduction and cleavage processes, through absence or low content of oxygen, through an abundance of carbondioxide and a relatively high content of nitrogenous and putrescible nutrient substances. The mud of this zone is frequently rich in ferrous sulphide.

We have no large streams of polysaprobiotic character over any great distance; even the river "Wupper" is not polysaprobiotic.

II - In the zone of the mesosaprobia, we distinguish sections with strong and/or weak mesosaprobiotic character. In the former of the two, self-purification seems to take place more aggressively than in the latter.

From the biological viewpoint, the first part of this zone is characterized by the predominance of Schizophyceae and -- especially in moving water -- by a more or less great abundance of Eumecytes; Peridiniales are practically completely absent. Animal life may be rather fully developed and thus may attract fishes for feeding. The fishes are here subject to asphyxiation only infrequently. The numerical content of bacteria per cubic centimeter is still high and may run into hundreds of thousands.

Good examples for this formation are furnished in particular by contaminated ponds and ditches, especially of trickle fields.

In contrast to the symbioses described so far and in special consideration of the benthonic diatoms, we might designate the second part of the mesosaprobiotic zone as the formation of the Bacillariaceae, especially if we consider the dearth of varieties in the strong mesosaprobiotic zone. In addition to the diatoms, we find here in general, however, a rather wide classification of the vegetation, e.g., among the Chlorophyceae, so that on the whole given types do not necessarily predominate.

The number of bacteria developing on standard nutritive gelatine normally amounts to less than 100,000.

All mesosaprobia are resistant to a minor affluence of sewage. Many of the higher aquatic plants find, particularly beyond the weak mesosaprobiotic zone, adequate and often rather favorable conditions of vegtation. The progressive course of self-purification is as characteristic from the chemical as from the biological viewpoint. Aeration and production of oxygen through assimilation of carbon have made possible the inception of oxidation processes which favor the life of the coarser fauna as already mentioned. Especially in the strong mesosaprobiotic part, the oxygen content tends to decrease, however, in darkness or with a clouded sky but rises again and often beyond the saturation maximum under increased light.

Such decomposition products of protein as asparagin, leucine, and glycine (all characterized by NH_4- and CCOH-groups, i.e., decomposition and oxidation stages) appear to be widely present in this zone (but in great dilution which makes their demonstration rather difficult) as well as ammonia salts and -- when approaching the oligosaprobiotic formation -- nitrites and nitrates, the oxidation stages of ammonia.

When stored in flasks, normal water from this zone does not tend to putrefy but may form a minor supernatant layer under certain circumstances.

Normal effluent from the trickle fields which may be designated -- at least during the hot season -- as typically nitrous water should probably be counted in the weak mesosaprobiotic region.

III - The zone of the oligosaprobia is characterized by the termination of mineralization and all aggressive processes of self-purification are normally absent here. The biological organization is manifold. If they exist, Peridiniales reach typical development in a few representatives. Sensitive to sewage, Charales now begin to show themselves but polysaprobia are absent even in small amounts. The number of bacteria developing on standard nutritive gelatine usually is less than 1,000 per ccm unless erratic forms have been infused. The dearth of planktonic Schizomycetes is also characteristic. Given benthonic forms of this class may occur, however, typically in the organic matting of the banks.

Chemical analysis of the waters from this zone shows us that the consumption of permaganate is relatively low and that we find only traces of organic nitrogen. Determined in a suitable manner, the consumption of oxygen is minor. Measured by immersing a white disk, the transparency of the water is generally high in calm weather. The mud from this region is generally poor in reduction processes but may assume a mesosaprobiotic character. In general, mud that can be characterized as oligosaprobiotic, will probably not be widely distributed.

Since the rapid decomposition of organic substances no longer dominates the chemistry of this region, less obviously effective substances may have an influence on ecological composition, e.g., such minerals as determine the differing hardness of the waters. However, there do not exist as yet any complete observations on this, not even for Phanerogamia.

The waters of all the above zones nearly all show an alkaline reaction; those with an acid reaction we intend to describe similar to the above at some later time.

We intend to publish elsewhere the "Ecology of Animal Saprobia" which is in harmony with our system.

PHYSIOLOGICAL SYSTEM OF PLANT SAPROBIA

Within the individual zones, the organisms are arranged in accordance with the Engler System, except for some deviations in the flagellate group. The following listing is based only on our own observations from nature. Such organisms as we did not ourselves observe at the main source of their development, have not been taken into account even if pertinent notes on them existed in literature; those have been omitted also which have no biocenotic value for the present purposes on the basis of our present experience (e.g., Bacterium cellulosae Omelianski Mig., many pantotrophic bactteria and various phanerogamia).

Doubts on the exact place of classification of some of the organisms were resolved by allocating them to the less nutrient of the respective zones.

I. Polysaprobia

Schizomycetes
Spirillum tenue Ehrbg.
" serpens (O. F. Müller).
" Rugula (O.F. Müller).
" Undula Ehrbg.
" volutans Ehrbg.
Sphaerotilus natans Ktz.
" roseus Zopf
cf. Mesosaprobia
Zoogloea ramigera Itzigsohn.
Streptococcus margaritaceus Schröter.
Sarcina paludosa Schröter
Beggiatoa alba (Vaucher) Trevisan
" leptomitiformis (Mengh.) Trevisan.
" arachnoidea (Ag.) Rabenhorst.
Thiopolycoccus ruber Win.
Chromatium okenii (Ehrbg.) Perty.
" vinosum (Ehrbg.) Win.
" minutissimum Win.
Lamprocystis roseo-persicina (Ktz.) Schröter.
cf. Mesosaprobia

Schizophyceae
Arthrospira jenneri Stitz., when associated with Beggiatoa. cf. Mesosaprobia.

Euglenales
Euglena viridis (Schrank)Ehrenbg., when abundant.

Protococcales
Polytoma uvella Ehrbg.

II. Mesosaprobia

1. Strong Mesosaprobiotic

Schizomycetes
Sphaerotilus natans Ktz.
" roseus Zopf
when associated with mesosaprobiotic Bacillariaceae and when in part with Cladothrix-like ramification. cf. Polysaprobia.
Thiothrix nivea (Rabenhorst) Win.
Chromatium okenii (Ehrbg.) Perty
Lamprocystis roseo-persicina (Ktz.) Schröter
when associated with mesosaprobiotic algae. cf. Polysaprobia.
Thiospirillum sanguineum (Ehrbg.) Win.
Spirochaete plicatilis Ehrbg., is classed by us as animal.

Schizophyceae
Oscillatoria princeps Vaucher
" tenuis Ag.
" chalybea Mertens.
" putrida Schmidle.
" chlorina Kutz
" splendida Grev.
" brevis Ktz.
" formosa Bory.
Arthrospira jenneri Stitz. cf. Polysaprobia.
Phormidium uncinatum (Ag.) Gomont.
" autumnal (Ag.) Gomont.
" foveolarum (Mont.) Gom.

Cryptomonadales
Cryptomonas nordstedtii (Hansg.) Senn; probably = Cryptoglena coerulescens Ehrbg.

Euglenales
Euglena viridis var. lacustris Francé.
Lepocinclis ovum Ehrbg.
" texta (Dug.) Lemm.
Cryptoglena pigra Ehrbg.

Bacillariales
Hantzschia amphioxys (Ehrbg.) Grun.
Nitzschia palea (Kutz.) W. Sm. and their variety fonticola Grun.
Stauroneis acuta W. Sm.

Protococcales
Chlamydomonas de baryana Gorosch.
Spondylomorum quaternarium Ehrbg.
Stichococcus bacillaris Naeg F. confervoidea Hazen. cf. weak mesosaprobia.
Chlorella infusionum (Beyerk.).

Confervales
Ulothrix subtilis Kuetz. forma.
Stigeoclonium tenue Ktz. (delimitation of variety difficult) attenuates toward weak mesosaprobiotic zone.

Phycomycetes
Mucor, and Zygorhynchus group.
Apodya lactea (Ag.) Cornu - Leptomitus lacteus Ag.

Hemiascomycetes
Endoblastoderma salmonicolor Fischer & Brebeck and some Torula which probably belong here.

Euascomycetes
Fusarium aquaeductuum Lagerheim.

2. Weak mesosaprobiotic

Schizomycetes
Lampropedia hyalina (Ehrbg.) Schröter.
Cladothrix dichotoma Cohn.

Schizophyceae
Oscillatoria limosa Ag.
" antliaria Jurgens
Phormidium subfuscum Ktz.
Aphanizomenon flos aquae Ralfs.

Chrysomonadales
Chrysosphaerella longispina Lauterb
Synura uvella Ehrbg., when associated with Closterium acerosum, Brachionus, Rotifer, actinurns and isolated specimens of Euglena viridis. cf. Oligosaprobia

Cryptomonadales
Cryptomonas crosa Ehrbg.
" ovata "

Euglenales
Euglena acus Ehrbg.
" spirogyra Ehrbg.
" oxyuris Schmarda.
" deses Ehrbg.
" pisciformis Klebs
" quartana Moroff.
" tripteris (Duj.) Kl.
" velata Klebs.
Phacus caudata Hübner.
Trachelomonas hispida Stein.
" volvocina Ehrbg.
Colacium vesiculosum (Ehrbg.) Stein

Peridiniales
Ceratium tetraceros Schrank, occurs also associated with Lamprocystis, Chromatium okenii.

Bacillariales
Melosira varians Ag. (preferred mineralized organic substance).
Stephanodiscus hantzschianus Grun
" " var. pusillus Grun.
Diatoma vulgare Bory.
Synedra Ulna var. splendens (Ktz.) J. Brun.
" actinastroides Lem.
" radians (Ktz.) Grun.
" vaucheriae Ktz.
Microneis minutissima (Ktz.) Cleve.
Navicula brebissonii Ktz.
" radiosa Ktz.
" cryptocephala Ktz.
" rhynchocephala Ktz.
" cuspidata Ktz.
" mesolepta Ehrbg.
" amphisbaena Bory.
" ambigua Ehbg.
" atomus Naeg.
Stauroneis phoenicenteron Ehrbg.
Gomphonema tenellum W. Sm.
" olivaceum Ktz.
" parvulum Ktz.
Rhoicosphenia curvata (Ktz.) Grun.
Nitzschia parvula W. Sm.
" communis Rabh.
" stagnorum Rabh.
" dissipata (Ktz.) Grun.
" acicularis (Rabh). W. Sm.
Surirella ovalis Breb. var. ovata = S. ovata Ktz., also var. minuta and angusta.

Conjugatae
Closterium acerosum Ehrbg.
" parvulum Naeg.
" moniliferum Ehrbg.
" leibleini Ktz.
Cosmarium botrytis Menegh.
Spirogyra crassa Ktz.
" porticalis (Vauch.) Cleve.

Protococcales
Carteria cordiformis Dill.
Chlamydomonas ehrenbergi Gorosch.
" brauni Gorosch.
" reinhardi Dang.
" kuteinikowi Gorosch.
" reticulata Gorosch.
Chlorogonium euchlorum Ehrbg.
Gonium sociale (Dng.) Warm. = Gonium tetras A. Br.
Stichococcus bacillaris Naeg; cf. strong Mesosaprobia.
Chlorococcum botryoides Rabh.
Pediastrum boryanum (Turp.) Menegh., especially when numerous young specimens exist.
Rhaphidium polymorphum var. aciculare (A.B.) Rab.
Scenedesumus auadricauda (Turp.) Bréb.
" acuminatus (Lagh.) Chodat
" obliquus (Turp.) Ktz.
" bijugatus (Turp.) Ktz.
especially when numerous young specimens exist.
Selenastrum bibraianum Reinsch.
Dictyosphaerium pulchellum Wood.
" ehrenbergianum Naeg.
Chlorosphaera limicola Beyrk.

Confervales
Ulothrix subtilis (Ktz.); cf. Oligosaprobia.
Conferva bombycina (Ag.) Wille.
Microthamnion kuetzingianum Naeg.
Oedogonium species.
Cladophora crispata Ktz.
Vaucheria sessilis (Vauch.) D.C.

Florideae
Hildebrandia rivularis (Liebm.) Bréb.

Monocotyledoneae
Holodea (Elodea) canadensis R. & Mchx.
Lemna minor L.
" polyrhiza L.

Dicotyledoneae
Ceratophyllum demersum L., when in certain forms of growth.

III. Oligosaprobia

Schizomycetes
Chlamydothrix ochracea (Ktz.) Mig.
Gallionella ferruginea Ehrbg.
Crenothrix polyspora Cohn.
Clonthrix fusca Roze.

Schizophyceae
Dactylococcopsis rhaphidioides Hansg.
Coelosphaerium kuetzingianum Naeg.
Gomphosphaeria lacustris Chodat.
Microsystis incerta Lemm.
Clathrocystis aeruginosa (Ktz.), Henfrey and other Microcystis-varieties.
Merismopedia glauca (Ehrbg.) Naeg.
" convoluta Bréb.
Oscillatoria anguina Bory.
" rubescens D. C.
" agardhii Gom.
Phormidium inundatum Ktz.
" papyraceuum (Ag.) Gom.
Microcoleus subtorulosus (Bréb.) Gom.

Anabaena flos aquae (Lyngb.) Bréb.
" spiroides Kleb.
Glaucothrix gracillima Zopf.
Calothrix parietina (Naeg.) Thuret.

Chrysomonadales
Chromulina rosanoffii Woron.
Mallomonas acaroides Perty.
" producta (Zach.) Iwanoff.
Synura uvella Ehrbg.; cf. mesosaprobia.
Uroglena volvox Ehrbg.
Dinobryon species.

Euglenales
Euglena oblonga Schmitz.
" geniculata (Duj.) Schmitz.
" minima Francé.
Phacus longicauda (Ehrbg.) Duj.
" pleuronectes Nitzsch.
" parvula Klebs.
" pyrum (Ehrbg.) St.

Peridiniales
Gymnodinium palustre Schilling.
Ceratium hirundinella O.F. Müll.
Peridinium minimum Schilling.
" quadridens Stein.
" cinctum Ehrbg.
" tabulatum Clap. & Lachm.
" berolinense Lemm.
" bipes Stein.
Gonyaulax apiculata (Pen.) Entz.

Bacillariales
Melosira ambigua O. Müll.
" granulata (Ehrbg.) Ralfs.
" italica Ktz.
" binderiana Ktz.
" crenulata Ktz.
" arenaria Moore and other species
Cyclotella meneghiniana Ktz.
" kuetzingiana Thw.
" comta (Ehrbg.) Ktz.
Tabellaria flocculosa (Roth) Ktz.
Meridion circulare Ag.
Fragilaria virescens Rulfs.
" construens (Ehrbg.) Grun.
" mutabilis (W. Sm.) Grun.
Asterionella formosa Hass.
Synedra acus Ktz.
Syedra ulna (Nitzsch) Ehrbg. and varieties
Eunotia arcus (Ehrbg.) Rabh.
Achnanthes exilis Ktz.
Navicula mesolepta Ehrbg.
" viridis Ktz.
" maior Ktz.
" gibba Ehrbg.
" dicephala W. Sm.
" inflata Ktz.
" iridis Ehrbg.
" limosa Ktz.
" gastrum Ehrbg.
" hungarica Grun.
" perpusilla Grun.
" viridula Ktz.
" clausii Gr.
Pleurosigma attenuatum (Ktz.) W. Sm.

Gomphonema acuminatum Ehrbg.
" capitatum Ehrbg.
" constrictum Ehrbg.
" angustatum Ktz.
Cymbella ehrenbergii Ktz.
" cistula (Hempr.) Kirchn.
" lanceolata (Ehrbg.) Kirchn.
Encyonema prostratum Ralfs.
" ventricosum Ktz.
Amphora ovalis Ktz.
Epithemia turgida (Ehrbg.) Ktz.
" sorex Ktz.
" zebra (Ehrbg.) Ktz.
Rhopalodia gibba (Ktz.) O. Müller
Bacillaria paradoxa Gmelin
Nitzschia sigmoidea (Ehrbg.) W. Sm.
" linearis (Ag.) W. Sm.
" vermicularis (Ktz.) Grun.
" vitrea Norman.
Cymatopleura elliptica (Bréb) W. Sm.
" solea (Bréb) W. Sm.
Surirella biseriata Bréb.
" splendida Ktz.

Conjugatae
Closterium lunula Ehrbg.
" dinanae Ehrbg.
" ehrenbergii Menegh.
" areolatum Wood.
Staurastrum tetracerum Ralfs.
Spirogyra irregularis Naeg.
" nitida (Dillw.) Linck.
" gracilis Ktz.
Mougeotia genuflexa (Dillw.) Ag.

Protococcales
Chlamydomonas angulosa Dill.
" intermedia Chod.
" longistigma Dill.
" pisiformis Dill.
" variabilis Dang.
Eudorina elegans Ehrbg.
Pandorina morum Bory.
Volvox globator L.
Carteria obtusa Dill.
Lobomonas francei Danj.
Pteromonas alata (Cohn) Seligo
Phacotus lenticularis Stein.
Tetraspora gelatinosa (Vauch.) Desv.
" explanata Ag.
Dimorphococcus lunatus A. Br.
Rhaphidium polymorphum Ktz.; parallel to Mesosaprobia.
Richteriella botryoides (Schmidle) Lemm.
Protococcus botryoides (Ktz.) Kirchn.
Pediastrum duplex Meyen.
" kawraiskyi Schmidle.
" tetras (Ehb.) Ralfs.
" Rotula (Ehb.) A. Br.
Actinastrum hantzschii Lagerh.
Coelastrum microporum Naeg.
" reticulatum (Dang.) Senn.
Sphaerocystis schroeteri Chod.
Hydrodictyon utriculatum (L.) Lagerh.
Botryococcus braunii Ktz.

Confervales

Ulothrix variabilis Ktz.
" subtilis var. variabilis (Ktz.) Kirchn.; cf. Mesosaprobia.
" zonata (Web. & Mohn) Ktz.
Draparnaldia glomerata (Vauch.) Ag.
" plumosa (Vauch.) Ag.
Chaetophora elegans (Roth) Ag.
Bulbochaete setigera Ag.
Coleochaete pulvinata A. Br.
Rhizoclonium hieroglyphieum (Ag.) Ktz.
Cladophora glomerata Ktz.
Vaucheria species.

Florideae
Lemanea torulosa (C. Ag.) Sirodot
Batrachospermum moniliforme Roth.

Bryophyta
Fontinalis antipyretica L.
Amblystegium riparium Schimp.

Pteridophyta
Salvinia natans All.
Isoetes lacustris L.

Monocotyledoneae
Potamogeton pectinatus L.
" crispus L.
Lemna trisulca L.

Dicotyledoneae
Nuphar lutenum Sm.
Nymphaea alba L., particularly the former is resistant to a great volume of sewage but does not indicate it.

In addition to the Oligosaprobia listed here, there are many others but these are less important for the evaluation of water.

LITERATURE REFERENCES

1. Kolkwitz and Marsson: Reports of the Royal Institute for Water Supply and Sewage Disposal, No. 1. 1902.

2. Lindau, Schiemenz, Marsson, Elsner, Proskauer and Thiesing: Hydrobiological and Hydrochemical Investigations on the Collector Systems of the Bäke, Nuthe, Panke and Schwärze. Quarterly for Forensic Medicine and Public Hygiene. Vol XXI, 1901, supplement.

3. Kolkwitz and Marsson: Principles for the Biological Evaluation of Water from Its Flora and Fauna. Reports of the Royal Institute for Water Supply and Sewage Disposal, No. 1, 1902.

4. Marsson: Flora and Fauna in Some Sewage Treatment Installations of Berlin and Their Significance for the Purification of Municipal Sewage. Ibid., No. 4, 1904.

5. Kolkwitz: Biological Self-Purification of Water in Nature and Mycology and Treatment of Sewage. Lafar, Manual of Technical Mycology, Vol. III, Chap. 14/15, 1906.

Further literature references and historical data will be found in these publications and further issues of the Institute.

Reproduced With Permission From:
INDUSTRIAL AND ENGINEERING CHEMISTRY
23(1931) : 75-78

EFFECT OF SUNLIGHT AND GREEN ORGANISMS* ON RE-AERATION OF STREAMS

Willem Rudolfs and H. Heukelekian

New Jersey Agricultural Experiment Station, New Brunswick, N. J.

The diurnal changes of dissolved oxygen in running streams were studied. The oxygen increased rapidly during the morning hours, reaching a maximum in the afternoon and a minimum at the early morning hours just before sunrise. The variations were similar in a tidal section of the Delaware, but were somewhat affected by the changes in tides.

Laboratory experiments with river water containing green algae showed that small temperature changes had practically no effect, the dissolved oxygen increasing or decreasing depending upon light and darkness. Direct sunlight was not an important factor, because increases were equally great with diffused light and with direct sunlight. The dissolved oxygen in water containing large quantities of blue-green and green algae could be decreased from supersaturation to 17 per cent saturation by placing the water in darkness, and could also be increased to 282 per cent saturation by subjecting it to diffused light. Changes in pH values followed changes in oxygen saturation. Under similar conditions the oxygen dissolved could be decreased by half when the number of organisms was decreased by half. Sampling during the afternoon of polluted rivers containing green organisms leads to erroneous results. In stream-pollution surveys all factors must be taken into consideration.

---

* Received September 27, 1930. Presented by Willem Rudolfs before the Division of Water, Sewage, and Sanitation at the 80th Meeting of the American Chemical Society, Cincinnati, Ohio, September 8 to 12, 1930.
Journal Series paper of the Department of Sewage Research, New Jersey Agricultural Experiment Station, New Brunswick, N.J.

Organisms containing the green pigment chlorophyll are capable of synthesizing complex organic compounds from carbon dioxide and water and giving off oxygen. This process, photosynthesis, takes place only in the presence of light. The first product of the process is presumably formaldehyde, from the condensation of which sugars and starches are formed. Aquatic plants capable of synthesizing their own food in this manner, and encountered in the streams, are mostly algae.

Some of the factors that influence photosynthesis are light intensity, temperature, partial pressure of carbon dioxide, and aeration. Light, especially the ultra-violet rays, stimulates the growth and activities of the green and blue-green algae. In turbid waters the penetration of light is reduced and hence photosynthesis is reduced. On the other hand, in a clear stream penetration of light is much greater and photosynthesis can take place at considerable depths.

The presence of carbon dioxide is also essential for photosynthesis. This may be replenished from the air or from the carbon dioxide produced as a result of respiration and bacterial activity. In the absence of free carbon dioxide plants may utilize the bicarbonates.

If conditions are favorable for photosynthesis, the oxygen content of the water will increase sometimes far beyond saturation. During the dark hours oxygen is consumed and carbon dioxide given off. During daylight the process is reversed. Actually during the day, then the oxygen concentration in a stream is the balance between these two processes -- the rate at which oxygen is liberated by the activities of green organisms and the rate at which it is consumed by respiration and oxidation. During the night, however, there will not be any liberation of oxygen, but the consumption will go on unabated with the result that the oxygen saturation will be lower. If the pollution in a stream is relatively great, the consumption of oxygen will overbalance its production and there will be depletion of oxygen. If, on the other hand, the pollution is slight, there may be a supersaturation of oxygen.

The green algae are most abundant in water during the summer. Their development follows the curve of the temperature of water, and maximum growth occurs in July and August. The optimum temperature for their growth is between 15° and 25° C., although some species can tolerate extreme heat or cold. The blue-green algae are also abundant during the summer months, although their maximum growth often occurs a little later in the season and some of them can tolerate somewhat higher temperatures than the green algae.

Although these changes in algal growth and the subsequent changes in dissolved gases in the water have been known for a long time, actual observations and determinations of diurnal changes in running streams have been reported only in a few instances. An observation made on the Illinois River (1909) was reported without much comment. Butcher, Pentilow, and Woodley (2) have given detailed studies on two polluted rivers in England. The studies by Birge and Juday (1) on the inland lakes of Wisconsin, although dealing with the changes taking place, were not concerned with running river water. Duvaux (4) and Duval and Dumarand (3) have discussed the mechanism and the rate of reaction changes in connection with the gaseous exchange of submerged aquatic plants. Moore (5) has published some observations on certain marine organisms in reaction to light, and Saunders (6) has published a note on photosynthesis and hydrogen-ion concentration. Several other investigators have dealt more remotely with the subject in hand, but none made an attempt to correlate the changes observed in the laboratory with actual conditions in streams.

It was with the purpose of evaluating the role of these green organisms in the re-aeration of the Delaware, Connecticut, and Raritan rivers that these studies were undertaken, but only some results obtained with the Delaware River water are here presented.

METHODS

Data obtained by sampling the river at definite places for 24-hour periods included temperature, dissolved oxygen, biochemical oxygen demand, and B. coli. Samples were taken in the middle and two quarter points of the river at mid depth. For the laboratory experiments large samples were obtained from different points in the Delaware River. Some samples were exposed to light or kept in the dark in open containers with uniform depth. Others were distributed into glass-stoppered bottles, which were immersed in a water bath and the temperature regulated with hot and cold water. At frequent intervals (1 to 1-1/2 hours) the temperature and dissolved oxygen content were determined. All transfers of the water were made by a siphon immersed under water to avoid bubbles. Analyses were made to complete 24-hour cycles; time is given as Eastern Standard Time. Analyses made according to the standard A.P.H.A. methods.

EFFECT OF SUNLIGHT ON RUNNING STREAMS

The dissolved oxygen determinations obtained in the tidal section of the Delaware River below Trenton, N. J., are given in Figure 1. The dissolved oxygen increased rapidly during the morning hours, with a maximum between noon and 4 p.m. During this period the tide was outgoing, so that the pollution, which is discharged by Trenton and the cities below, had no effect. With the turn of the tide the dissolved oxygen decreased rapidly during the next few hours, with the decrease slowing up when the maximum flood tide approached. During daylight hours the dissolved oxygen increased rapidly with outgoing tide, but during the night with outgoing tide it decreased still further until the lowest was reached at about 3 a.m. As daylight increased the dissolved oxygen increased. The temperature of the water was constant between 8 a.m. and 6 p.m., and in spite of the decrease in temperature the dissolved oxygen decreased rapidly during the night. This is contrary to the solubility of oxygen at

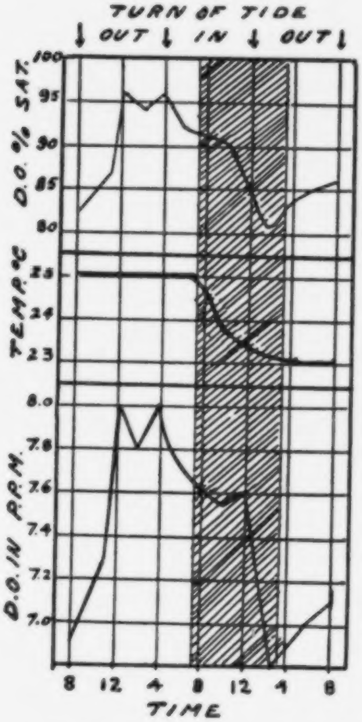


Figure 1 - Dissolved Oxygen in the Tidal Section of Delaware River.

different temperatures; an increase in dissolved oxygen rather than a decrease could be expected. These changes were not peculiar to the water in the tidal section. For example, the results obtained during daylight hours in the river about 50 miles above Trenton (the river is subject to tide up to Trenton) 2 days previous are given in Figure 2. The temperature fluctuation during the day was 1° C. The rapid increase in dissolved oxygen continued until a maximum was reached at 3 p.m. The increase and decrease in saturation occurred irrespective of any small temperature fluctuations. Similar results were obtained in a number of instances, which will be published elsewhere. This paper deals mainly with some of the factors which seem to be responsible for the fluctuations.

A set of results obtained with Delaware River water, brought to the laboratory, is given in Figure 3. The experiment was conducted on August 6, 1929, and the days following. The dissolved oxygen decreased gradually until 3:15 a.m., after which it began to rise slowly but did not reach the original value. The dissolved oxygen was practically constant during the afternoon with a constant temperature, decreased with a rapidly decreasing temperature, followed again by a slow rise in dissolved oxygen in spite of a further decrease in temperature. The decrease of dissolved oxygen at night, in spite of the decreasing temperature, was undoubtedly due to the consumption of oxygen by microorganisms in the process of respiration and decomposition. The rise in dissolved oxygen in the early morning hours before the action of light became effective was probably due to the further decrease in temperature, but the continued rise during the rest of the day must be due to the activities of the green organisms. In this case re-aeration was confined to the green organisms, since wind and rippling of the surface of the water were eliminated.

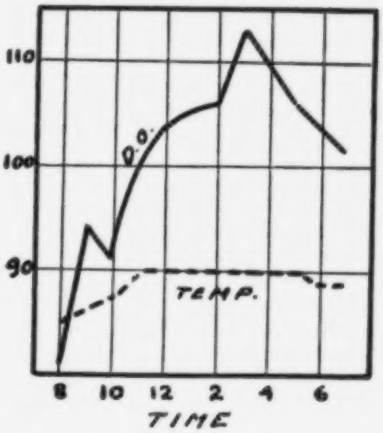


Figure 2 - Dissolved Oxygen during Daylight above Trenton, N. J.

The samples of water were kept in diffused light, and it was thought that the re-aeration due to the organisms might have been more intense if the water was kept in direct sunlight. The samples were exposed for 6 hours to direct sunlight and analyses made at intervals. An exposure of 1-1/2 hours increased the dissolved oxygen from 7.2 to 7.5 p.p.m., while the temperature increased from 20° to 24.5° C. No further increase in dissolved oxygen occurred, but the temperature of the water continued to rise so that the dissolved-oxygen saturation increased from 80 to 93 per cent. Thus the intensity of the light (direct sunlight with higher temperature as compared with light from an overcast sky) did not increase the degree of re-aeration, but hastened the time in which maximum oxygen production was possible.

Two other series of samples were subjected to light and darkness, while another series was kept in the dark. Those exposed to the light increased in dissolved oxygen while the temperature decreased; those kept in the dark decreased gradually in dissolved oxygen. The difference caused by the action of light amounted to 15 per cent.

Other samples were placed in stoppered glass bottles and submerged in water. In these instances no surface aeration could take place and the light had to penetrate not only a depth of 6 inches (15 cm.) of water but also the glass. The results are shown in Figure 4. An increase in dissolved oxygen took place until between 7 and 8 p.m., when a maximum was reached of 9.5 p.p.m. During the afternoon the saturation increased to 112 per cent, decreasing to about 100 per cent at 4:30 a.m. and again increasing to 118 per cent at 11 a.m. In these instances the oxygen liberated could not escape from the bottles, which accounts for the saturated condition even in the early morning hours. The water kept in the dark decreased from 8.4 to 7.5 p.p.m. in a straight line, while the saturation decreased from 101 to 90 per cent as compared with the increase to 118 per cent.

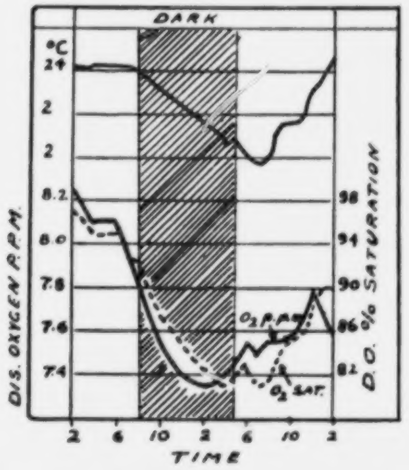


Figure 3 - Dissolved Oxygen in Delaware River Water as Tested in Laboratory.

Experiments made with distilled water to find out whether daily fluctuations took place when exposed to similar conditions in open beakers and stoppered bottles showed that the dissolved oxygen of the water did not increase in the dark and decreased slightly in the light.

The most abundant green organisms were Closterium, Pleurococcus, Eremosphora, Scenedesmus, Protococcus, Raphidium, and Botrycoccus. The number of algae were as high as 5200 per cc.

DIURNAL CHANGES IN WATER CONTAINING GREEN ALGAE

The diurnal changes in dissolved oxygen are similar from day to day, except that the dissolved oxygen may gradually increase. During the summer of 1930 laboratory experiments were conducted in an effort to determine the relation of the number of organisms to the chemical changes taking place.

Large samples of water, densely green with organisms, were collected from a creek tributary to the Delaware, receiving the effluent from some small sewage-disposal plants. The water was placed in carboys and subjected to alternating light and darkness for varying lengths of time.

The dissolved oxygen results of a part of the experiments, together with the changes in pH values, are given in Figure 5. The water when collected had a dissolved-oxygen content of 12.9 p.p.m. or a saturation of 154 per cent. After having been kept for 1 day in semi-darkness, the water was placed in an incubator for 18 hours, then placed in daylight for 10 hours, and so on. The dissolved oxygen dropped during the time the water was kept in semi-darkness from 12.9 to 2.2 p.p.m. and after having been placed in the incubator to 1.2 p.p.m. During the next 7 daylight hours the dissolved oxygen increased to 14.7 p.p.m., decreased again during the night, and rose the following day to 21 p.p.m. Again during the next dark period it dropped to 10.9 p.p.m., from which it rose in 8 hours to 23.8 p.p.m. or a saturation of 282 per cent. The water was then subjected to a prolonged period of darkness of 63 hours, after which time the dissolved oxygen dropped to 3.2 p.p.m. During these periods the temperature was kept constant in the incubator at 21° C. As soon as the water was put back into daylight, the dissolved oxygen increased in 9 hours from 3.2 to 15.4 p.p.m.

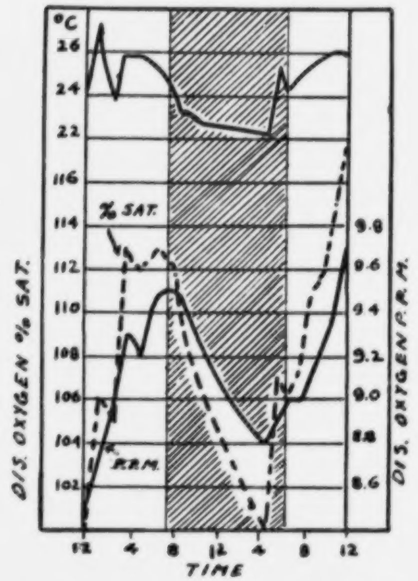


Figure 4 - Dissolved Oxygen in Samples Placed in Stoppered Bottles Submerged in Water.

The pH values fluctuated directly with the dissolved oxygen as may be seen from Figure 5. They rose by steps from 6.9 to 9.6+, while during the prolonged period of darkness this figure decreased to pH 7.1. The changes in carbon dioxide and carbonates were far less pronounced.

It is evident that the action of light has a cumulative effect, reaching under ordinary conditions its maximum at mid-day or thereafter. It seems to be more a time factor than merely an intensity effect, because the effects were the same when radiation was through glass or on cloudy days.

The numbers of blue-green and green organisms (photosynthetic) in the water were very high and mainly of the following species: Oscillatoria, diatoms (about six genera, particularly filamentous forms), Scenedesmus, and a few Microcytis, Pediastrum, and Desmids. Among the green flagellate protozoa Trachilomonas was most abundant. Most of the organisms were the blue-green Oscillatoria. The photosynthetic organisms slowly decreased during the 8 days of the experiment here reported -- namely, from 45,400 to 32,700 per cc. Bacterial eaters gradually increased.

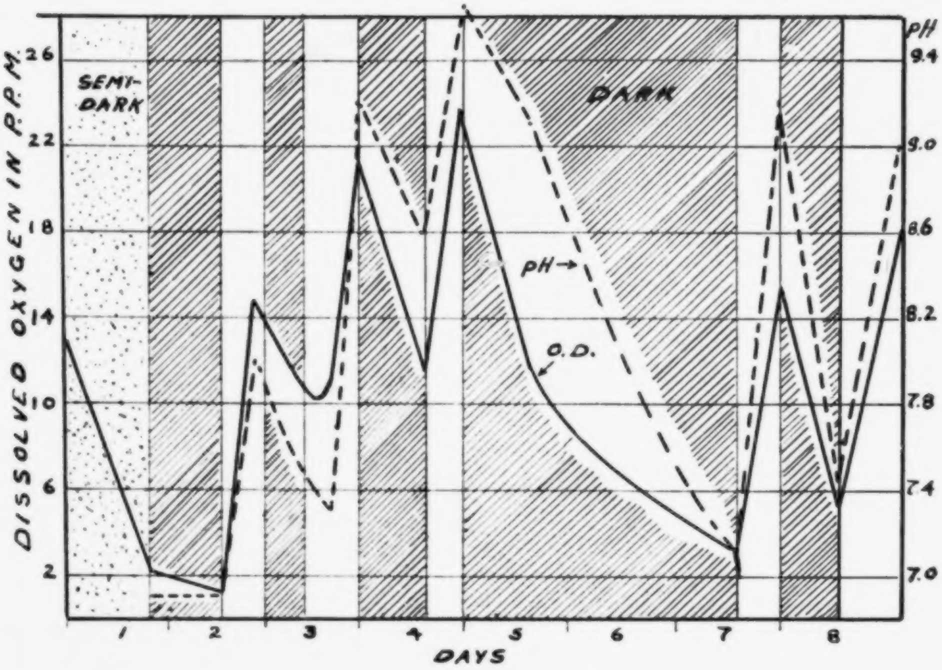


Figure 5 - Changes in Dissolved Oxygen and pH.

EFFECT OF CONCENTRATION OF ORGANISMS

The water with the large number of organisms was diluted by half with a mixture of river and tap water. The effect on the oxygen dissolved in the water was very pronounced, as may be seen from the following figures:

Time	Dissolved Oxygen	
Days	Concentrated P.p.m.	Diluted P.p.m.
1	14.7	10.4
2	21.0	10.0
3	23.8	11.1
6	15.4	7.4

In the "diluted" water there were approximately half as many organisms as in the "concentrated" water, while the dissolved oxygen was also approximately half.

DISCUSSION

In dealing with the pollution of a stream the role of re-aeration by green organisms must be properly evaluated. If the pollution is not excessive so that the production of oxygen is overbalanced by the consumption, a marked increase in dissolved oxygen will result. With a more heavily polluted stream the dissolved oxygen might show from day to day, when samples are taken in the afternoon, complete saturation leading to erroneous conclusions because the temporary condition in the afternoon is by no means the daily average condition. Moreover, these erroneous results are usually obtained in the spring and especially in July and August with low stream-flow conditions and higher temperatures. From the reported and other results obtained it is believed that results for dissolved oxygen have been interpreted as meaning far more than was actually warranted. In stream-pollution surveys several more factors must be taken into consideration. From the high pH values obtained during the afternoons the deduction could have been made that either the water was alkaline or that large quantities of strongly alkaline trade wastes had been discharged.

LITERATURE CITED

1. Birge and Juday, Wisconsin Geol. Natl. Hist. Survey, Bull. 22 (1911).

2. Butcher, Pentilow, and Woodley, Biochem. J., 21, 945, 1423 (1927); 22, 1035, 1478 (1928).

3. Duval and Dumarand, Compt. rend. soc. biol., 89, 398 (1923).

4. Duvaux, Ann. sci. nat., 9, 286 (1889).

5. Moore, Biochem. J., 4, Nos. 1 and 2 (1908).

6. Saunders, Proc. Cambridge Phil. Soc., 19, 24 (1920).

Reproduced With Permission From:
ILLINOIS NATURAL HISTORY SURVEY BULLETIN
19(1932) : 469-486

THE PLANKTON OF THE SANGAMON RIVER IN THE SUMMER OF 1929

Samuel Eddy

The Sangamon River, a small river in the central part of Illinois, has special interest to students of aquatic biology because it exhibits in a remarkable way the effects of the installation of a sewage treatment plant in alleviating pollution and at the same time the effects of the erection of a dam to impound water for municipal and industrial uses. The present study is an attempt to determine to what extent these effects are reflected by changes in the abundance of certain kinds of microscopic organisms, collectively called plankton, which live suspended in the water. As is well known, some kinds of plankton organisms, if present in sufficient numbers in reservoirs, may give disagreeable flavors to the water; other kinds may aid in the natural purification of polluted waters; and in streams and lakes generally plankton plays a role of more or less importance as food for larger organisms, including fishes. In our larger streams, such as the Rock River and the Illinois River, the plankton may be an important factor in fish production. In the Kaskaskia River, as an example of our smaller streams, the plankton is so scanty that it can have very little importance. The Sangamon River illustrates an intermediate stage in which the plankton is abundant enough to enter into the food chains of fishes to some extent but does not result in a yield of fishes appreciably greater than in the Kaskaskia. The writer's observations of plankton development in the upper part of the Sangamon from 1923 to 1929 were included in a general study on "Fresh-water Plankton Communities," submitted as a thesis in the Graduate School of the University of Illinois but not yet published. Further collections, made during the summer of 1929, showing the variety and abundance of organisms present at selected stations along the lower part of the river as well as the upper part, are reported in this paper.

The Sangamon River rises in McLean County and at first flows eastward into the northwestern part of Champaign County, where it turns southward and passes the villages of Foosland, Fisher, and Mahomet. It then flows in a general southwestward direction across Piatt County, passing Monticello, and across Macon County, passing Decatur. In Sangamon County, it receives its first large tributary, the South Fork, and passes Riverton and Springfield. It flows northward across Menard County, passing Petersburg. At its junction with Salt Creek it turns westward. It continues in a generally westward direction, forming the boundary between Mason County and Cass County, passing the village of Chandlerville, and finally emptying into the Illinois River about ten miles above Beardstown.

The length of the river is about 237 miles. The distance from the source to Decatur is about 103 miles, from Decatur to Springfield about 59 miles, and from Springfield to the mouth about 75 miles. The total drainage area is about 5,390 square miles, of which 1,940 square miles belong to Salt Creek, and 846 square miles belong to South Fork.

The current of the river at normal levels is never very great, since it flows through glacial till and occupies a well-worn valley. The river falls 120 feet in the first 10 miles and 300 feet in the balance of its course, or less than 2 feet per mile. The fall is far from regular, however, and there are many stretches where the gradient is very slight. The bottom is usually sand or fine silt, the latter predominating over most of the river.

Previous to 1923, no obstructions were encountered in the upper course of the river except several small ruined mill-dams. At Decatur a small dam raised the water level a few feet and held back a small supply for municipal purposes. Below this dam the city of Decatur discharged all its sewage, which usually was greater in volume than the water flowing over the dam. During times of low water, the sewage constituted the entire flow of the river below the dam, as all of the water above the dam was then diverted through the city water supply system. As a result of the pollution, the river below Decatur was devoid of normal aquatic life. Jewell (1920) reported no living organisms immediately below Decatur except those accustomed to conditions of pollution. Thirty miles below Decatur conditions were found to be improving, but even at Springfield, almost 60 miles downstream, the normal life was not completely restored. The writer was well acquainted with the river previous to 1920 and clearly remembers the extreme condition of pollution existing for at least 20 miles below Decatur. The bottom was covered with a thick layer of foul sludge, and the opaque water, which varied in color from inky black to milky white, depending on the season, contained large quantities of floating mouldy wastes.

At Springfield a second dam has obstructed the river for many years, raising the level of the water about six feet and forming a narrow pool that extends upstream several miles. Pollution below Springfield has been largely eliminated since the city's sewage treatment plant was put into operation, July 10, 1929. The wastes from Petersburg and Chandlerville are not sufficient to pollute the river to an appreciable extent.

In 1923 the city of Decatur, in order to increase its water supply, completed a large dam across the river just above the site of the old one, creating a lake one-half mile wide and 12 miles long. Later, in 1924, a sewage disposal plant was put into operation, handling one-third of the city wastes, and in 1928 the plant was enlarged to accommodate all the wastes. This relieved the extreme condition of pollution. The dredging of a new channel from Harristown for 20 miles downstream has aided by furnishing a new bed, free from the accumulated bottom sludge. These changes have resulted in a fairly clean stream, flowing through a lake-like reservoir in its upper region.

In the writer's previous study of the plankton of the upper river from 1923 to 1928, inclusive, weekly or semi-monthly collections were made from the river at Decatur and above. Very little plankton was found above Lake Decatur in those years. At Mahomet, about 50 miles from the source, no plankton forms occurred, though bottom organisms, especially diatoms and protozoans, occasionally appeared in the collections. The same was true at Monticello during the greater part of the year, but in mid-summer or early autumn, when the water was low, a scanty population of plankton organisms was found there. This was the first point in the course of the stream where plankton ever appeared. At Rhea's Bridge on the upper end of Lake Decatur, plankton was present in the water during most of the year but was never as abundant as at Lost Bridge, one mile above the dam, where many plankton species were abundant from March until December. During January and February the plankton of the lake was scanty and consisted chiefly of protozoans.

In 1929, trips were made in June, July, and September, for the purpose of collecting samples at intervals of about 20 miles over the entire river. The collections were made from bridges at or near Mahomet, Monticello, Lake Decatur, Harristown, Illiopolis, Riverton, Springfield, Petersburg, and Chandlerville. The method was to dip the water from mid-channel by lowering a ten-liter bucket on a rope. No stratification of plankton was noticed except at Lake Decatur, where the current was negligible and the plankton was much heavier near the surface. The principal collecting station on the lake was at Lost Bridge, and at this station a series of collections was made from bottom to surface and averaged. Each set of collections made at the other stations consisted of a 100-liter silk-net collection and a one-liter collection which was preserved with formalin and allowed to settle and then decanted in order to obtain the nannoplankton. The organisms were counted by the usual method in a Sedgwick-Rafter slide. All data were computed per cubic meter. The volume of the plankton in the silk-net collections was obtained by centrifuging for 3 minutes at 2000 revolutions per minute. The volume of the decanted plankton was not determined because of the large amount of silt present.

At the times of collecting, the river was 1-2 feet deep and 25-40 feet wide at Mahomet, the uppermost station, and 8-10 feet deep and 200-210 feet wide at Chandlerville, the lowermost station. The deepest portion from which collections were made was in Lake Decatur where the depth in the channel ranged from 10 to 18 feet. Below Decatur the river was quite shallow. It was 2-4 feet deep and 60-75 feet wide at the Harristown and Illiopolis bridges. At Riverton, after the union with the South Fork, the river was 4-6 feet deep and 100-114 feet wide. At the bridge north of Springfield the water (backed up by the dam) was 5-7 feet deep and 100-110 feet wide. At Petersburg the river was 100-150 feet wide and 7-10 feet deep in mid-channel.

The current, which was moderate at most places, was very slow in some stretches of the river and somewhat swifter in others with a greater fall. At Mahomet and Monticello, the current averaged about one-half mile per hour during the summer. In the main part of Lake Decatur no current could be detected. At Harristown and Illiopolis the current averaged about one mile per hour; at Riverton one-half mile per hour; and at Springfield just above the dam it was too slow to estimate. At Petersburg and Chandlerville it averaged a little more than one-half mile per hour.

The river level was slightly above normal when the plankton collections were made in June and July. The readings of the gage at the Decatur sewage disposal plant averaged 587.3 feet for June 25-27 and 587.6 feet for July 26-28. April and May were the highest months for the year 1929, the gage at the disposal plant reaching a maximum of 593.5 feet in those months. The lowest stage for the year was in September, although the gage readings on the dates of collections, September 10-12, averaging 584.0 feet, were not the lowest of the month. Thus the river was about 3-1/2 feet lower in September than it was when the June and July collections were made. The Decatur lake, however, did not fluctuate much, as the gage reading above the dam was 610.25 in June and July and 609.95 in September.

The temperature of the water at the time of collecting was about what would be expected under normal summer conditions. In June it ranged from 24° to 25° C. and in July from 25° to 28° C. No temperature data were obtained on the September trip, as the thermometer was broken in the field.

Hydrogen ion determinations were made at all stations and were found to run consistently about pH 7.6. This seemed normal for the river, as the readings agreed with those obtained by the writer in previous observations. On the upper river from 1923 to 1929, the readings in summer always ranged around pH 7.6, dropping to pH 7.0 or lower in winter.

Determinations of dissolved oxygen in the water at each station on the June trip were as follows: Mahomet 4.75 cc. per liter, Monticello 4.06, Decatur 4.62, Harristown 4.06, Illiopolis 4.62, Riverton 4.25, Petersburg 4.25, and Chandlerville 6.47. No determinations can be given for Springfield, as the June collections and data from that station were accidently lost. There was only a slight fluctuation in the amount of dissolved oxygen in the water at the various stations. At all points examined, the supply seemed sufficient for the support of abundant aquatic life.

A summary of the plankton collections is given in Table I, and the constituent organisms are listed in Tables II, III, and IV.

The general taxonomic composition of the plankton found in the river below Monticello was the same as that observed in most shallow lakes and larger streams of North America. Certain typical forms were conspicuous in their proper seasons, namely: two protozoans, *Codonella cratera* and *Ceratium hirundinella*; rotifers of the genera *Brachionus*, *Synchaeta*, *Polyarthra*, and *Keratella*; various cladocerans, particularly *Moina affinis*, *Daphnia longispina*, and *Bosmina longirostris*; and two copepods, *Diaptomus siciloides* and *Cyclops bicuspidatus*. A few bottom organisms, usually diatoms and protozoans, were often conspicuous in the plankton collections from the shallow portions of the river where the current could easily sweep them up from the bottom. They did not often appear in the collections from Lake Decatur but were quite common in the collections from the other stations, especially at Monticello, Harristown and Illiopolis.

The collections made in June showed no plankton at Mahomet and only a very scanty plankton at Monticello. The first heavy plankton occurred at Lost Bridge in Lake Decatur. Both the volume of the plankton and the number of species in the collections decreased downstream as far as Petersburg and Chandlerville, where the number of species increased slightly, though the volume of the plankton continued to decrease. Of the 50 species observed in the collections from the entire river in June, 36 appeared in Lake Decatur and 12 appeared farther downstream in relatively small numbers. Evidently a large amount of the downstream plankton owed its origin to the increase in Lake Decatur. Characteristic species which were most conspicuous in Lake Decatur and showed a decrease downstream were *Difflugia lobostoma*, *Codonella cratera*, *Brachionus angularis*, *Polyarthra trigla*, *Keratella cochlearis*, *Moina affinis*, *Daphnia longispina*, *Bosmina longirostris*, *Cyclops bicuspidatus*, and *Lysigonium (Melosira) granulatum*. Several other members of the plankton conspicuous in the lake did not appear at all below. The flagellates, *Trachelomonas volvocina* and *Euglena viridis*, decreased downstream to Petersburg and then started to increase. The only form that showed a decided increase below the lake was one of the algae, *Actinastrum hantzschi*.

The collections made in July showed that the plankton then had a distribution very similar to that of the preceding month. At Mahomet there was no plankton at all, and at Monticello it was very scanty. Of the 58 species found in the July collections, 47 first became abundant in Lake Decatur. Only 8 species occurred downstream which did not occur in the lake, and these were usually rare or inconspicuous, never forming an important part of the plankton. Many forms which were conspicuous in Lake Decatur showed a decided decrease below Decatur and apparently had their origin in the lake. Examples of these were the following: *Pandorina morum*, *Pleodorina illinoisensis*, *Codonella cratera*, *Difflugia lobostoma*, *Trachelomonas ensifera*, *Euglena viridis*, *Ceratium hirundinella*, *Eudorina elegans*, three species of the genus *Brachionus* and species of *Filinia* (*Triarthra*), *Asplanchna*, *Polyarthra*, *Synchaeta*, *Pedalia*, and *Trichocerca* (*Rattulus*), and *Lysigonium (Melosira) granulatum*. Only a few forms which decreased below Decatur showed a slight increase at Chandlerville. A peculiar feature was an increase in the number of *Cyclops bicuspidatus*, *Keratella cochlearis*, *Diaphanosoma brachyurum*, and *Brachionus angularis* at Harristown and Illiopolis. In general, the July plankton showed a decided decrease below Decatur. Just as in June, the lake apparently was acting as a reservoir, developing an abundant plankton which was then carried downstream and gradually thinned out in the lower river as the water was diluted by tributaries.

The September collections were made under somewhat different conditions from those in June and July, for the level of Lake Decatur was slightly below the crest of the dam, so that little or no water passed over, and the chief source of the water in the river below Decatur was the effluent from the city's sewage disposal plant. The current in the river was not as swift as at higher river levels, and under such conditions the larger tributaries, particularly the South Fork and Salt Creek, might be expected to add a small amount of plankton. While there still were no plankton organisms found at Mahomet, the plankton was more abundant at Monticello than previously. In Lake Decatur the collections were found to have a somewhat smaller volume and to include fewer species than previously. Downstream from the lake, however, many species, apparently originating in it, especially protozoans and algae, showed a steady increase in abundance; and several species additional to the lake list make their first appearance just below Decatur. Many conspicuous members of the plankton, particularly rotifers, appeared first at Riverton after the union with the South Fork and were abundant downstream from there. A decided increase in both abundance and species was noted at Springfield, which may in part be due to slack water above the dam. A further increase at Petersburg indicated that other conditions were favorable for greater plankton production.

Only a few species abundant in Lake Decatur showed a tendency to decrease rather than increase downstream. These were *Difflugia lobostoma*, *Codonella cratera*, *Brachionus angularis*, *Keratella cochlearis*, and some of the cladocerans and copepods. Of the 66 species found in the September collections, only 25 occurred in Lake Decatur, and 33 species occurred in the downstream collections which did not appear in the lake. Many of the latter showed a tendency to increase downstream. The increase was marked at Springfield and Petersburg, indicating that the lower river was maintaining a plankton population due in part to the low water stage and the reduction of the current. Such conditions in the river approach lake conditions, the waters remaining longer in the pool-like stretches. Since no water was then coming directly downstream from the lake, all the water in the river was effluent from the sewage disposal plant. Agersborg (1929) found this effluent to be teeming with annelids, rotifers, copepods, protozoans and algae, and states that these are organisms such as live in small ponds, though failing to mention any species which are typical of either clean-water or sewage plankton. It is doubtful if many, or any at all, of the lake forms survive passage through the sewage disposal plant, comprising as it does both sand filters, tanks and Dorr separators; and still more doubtful that there is any important development of additional plankton species until after the passage through the plant is completed.

Sphaerotilus natans, although very abundant in the sewage disposal plant, did not appear at any time in the collections. It is probable that the origin of many of the downstream plankton species at this period was in the quieter stretches of the river, which were seeded by the plankton originating in the lake at times when the water was passing over the dam.

The question often arises whether the plankton in a given part of a stream is developed there under local conditions or whether it is carried down from upstream. This survey indicates that, at times, part of the plankton, at least, is carried downstream. Wiebe (1928) and the Minnesota State Board of Health (1928) show that clean plankton from upstream is carried through polluted areas in the Mississippi below Minneapolis and St. Paul. In the Sangamon the downstream decrease observed in June and July may be due partly to the fact that local development was not sufficient to counterbalance the dilution from tributaries. In September, when the low stage of the water cut off the direct supply from the lake, local conditions downstream became more favorable for the development of plankton. When the current becomes very slow, the development of plankton becomes local and is governed by local conditions. If the current averaged one-half mile per hour, the time required for water to flow from the source to the mouth would be about 20 days. However, at normal stages the river has many pool-like stretches which retard part of the water, and rough estimates of the period of detention in the lake at Decatur range from two weeks to two months, depending on the river level. Thus the water remains in the lake at least twice as long as in the rest of the river. The water and the plankton it bears as it flows over the dam at Decatur will ordinarily be in the neighborhood of Chandlerville about a week later. In this way, at normal levels, there is a continual stream of water carrying plankton from the lake and passing downstream. Even though the downstream conditions are not favorable for plankton development, it seems possible for the water to retain part of the original plankton load for a week or more, so that a series of collections made at various points in the lower course of the river do not represent the development of plankton at each point but give glimpses of various stages of senescence as the plankton moves away from its source. It may be possible that the plankton observed downstream in September had originated from the lake when the water was still flowing over the dam, and that it was still progressing downstream. This, however, would hardly explain the origin of the plankton observed in the river immediately below Decatur.

No evidence of the former pollution was observed in the plankton of the Sangamon River. No pollutional organisms were found at Harristown, about eight miles below Decatur, where Jewell ten years earlier had found the plankton to be characterized by *Sphaerotilus natans*, nematodes, ciliates, and creeping *rotifers*, with desmids and phytoflagellates common when the water was high. In this area in 1929 the plankton was typical of clean water and was characterized by *Codonella cratera*, *Polyarthra trigla*, rotifers of the genus *Brachionus*, *Cyclops bicuspidatus*, cladocerans, and *Lysigonium (Melosira) granulatum*. Jewell found the dissolved oxygen usually low in that part of the river, especially during periods of low water. The determinations of dissolved oxygen made in 1929 showed an abundant supply. The sludge which was formerly so abundant had nearly disappeared, the water was clear, and a number of fishes were observed.

Very little is known regarding the former condition of the plankton below the polluted part of the river. Jewell's studies in 1918-1919, which extended only as far as Springfield, showed that the influence of pollution had partly ceased there and that a few typical clean-water plankton organisms were present in the river at that point. The abundant clean-water plankton now found in that part of the stream, including many more species than were reported by Jewell, indicates that the plankton population in the lower river is much greater than formerly, and this is due, no doubt, to the creation of the lake at Decatur and to the removal of the pollution barrier.

BIBLIOGRAPHY

Agersborg, H. P. K. 1929. The Biology of Sewage Disposal. A Preliminary Study. Trans. Am. Micros. Soc., Vol. 48, pp. 158-180.

Greeley, S.A., and Hatfield, W.D. 1928. The Sewage Disposal Works of Decatur, Illinois. Proc. Am. Soc. Civil Eng., Vol. 92, pp. 2237-2286.

Jewell, M.E. 1920. The Quality of Water in the Sangamon River. Ill. State Water Surv. Bul. No. 16, pp. 230-246.

Minnesota State Board of Health. 1928. Report of the Investigation of the Pollution of the Mississippi River from Minneapolis to LaCrosse. Metropolitan Drainage Commission of Minneapolis and St. Paul, 2nd Annual Report, pp 90-102.

Wiebe, A.H. 1927. Biological Survey of the Upper Mississippi River with Special Reference to Pollution. U.S. Bur. Fish. Bul., Vol. 43, pp. 137-167.

TABLE I.

SUMMARY OF PLANKTON COLLECTIONS, SANGAMON RIVER, 1929.

Station	Volume (cc. per cubic meter)			Number of species represented		
	June 25-27	July 26-28	Sept. 10-12	June 25-27	July 26-28	Sept. 10-12
Monticello..........	.08	.08	.05	9	8	16
Lake Decatur.......	15.20	9.50	9.60	36	47	25
Harristown.........	6.00	8.00	10.00	22	34	27
Illiopolis............	9.00	8.00	8.40	23	31	26
Riverton............	3.00	3.90	3.60	17	35	35
Springfield.........	....	.80	12.60	..	28	36
Petersburg..........	2.20	.40	17.00	22	22	39
Chandlerville.......	.50	.30	6.00	24	22	30

TABLE II.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, JUNE 25-27, 1929.

Italics indicate decantation collections.

Organisms	Monticello	Lake Decatur	Harristown	Illiopolis	Riverton	Petersburg	Chandler-ville
PROTOZOA							
Difflugia lobostoma Leidy	*86,820*	*100,000*	*86,658*	*60,000*	*25,000*	*22,000*	*20,000*
Trachelomonas ensifera Daday	*43,000*						*888,000*
Euglena sp.	*40,000*	*50,000*					*660,000*
Codonella cratera (Leidy)	*42,000*	*6,000,000*	*288,860*	*280,000*	*290,000*	*440,000*	*60,000*
Tintinnidium fluviatile Stein		*750,000*			*14,000*	*11,500*	*220,000*
Phacus longicaudus (Ehr.)		500		580		400	
Ceratium hirundinella O.F.M.		1,000					2,500
Euglena oxyuris Schmarda		*50,000*		1,100		800	
Trachelomonas volvocina Ehr.		*50,000*	*28,850*	*27,000*	*28,000*	*20,000*	*84,000*
Euglena viridis Ehr.		*100,000*	*28,000*	*55,000*	*16,000*	*22,200*	*110,000*
Dinobryon sertularia Ehr.		15,000	15,000	11,600			250
Centropyxis aculeata Stein			2,000	2,200	1,000	1,600	
Arcella vulgaris Ehr.			*25,000*	*28,000*	550	800	
Trachelomonas hispida (Perty)				*25,000*			
Pleodorina illinoisensis Kofoid					510		250
Eudorina elegans Ehr.						2,400	500
Euglena acus Ehr.							120
Pandorina morum Bory							*110,000*
ROTATORIA							
Filinia longiseta (Ehr.)	240	10,000					
Brachionus angularis Gosse	200	10,000	500	580	500	700	2,500
Brachionus capsuliflorus Pallas		3,000					
Brachionus calyciflorus Pallas		1,500					
Brachionus budapestinensis Daday		15,000				400	
Synchaeta pectinata Ehr.		500				1,200	
Brachionus patulus O.F.M.		500					
Asplanchna sp.		15,000					500
Polyarthra trigla Ehr.		90,000	15,000	8,700	1,100	12,000	1,000

TABLE II—Concluded.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, JUNE 25-27, 1929.

Italics indicate decantation collections.

Organisms	Monticello	Lake Decatur	Harristown	Illiopolis	Riverton	Petersburg	Chandler-ville
Keratella cochlearis (Gosse)		50,000	2,500	1,200	1,080	8,000	3,700
Lecane spinifera (Western)		1,000					
Rotaria neptunia (Ehr.)		250					250
Pedalis mira (Hudson)			1,000				
CLADOCERA							
Moina affinis Birge		30,000	500				
Diaphanosoma brachyurum (Liéven)		2,500	15,000	2,000			
Daphnia longispina (O.F.M.)		15,000	1,000	1,100			
Bosmina longirostris (O.F.M.)		10,000	1,000	5,800			2,500
Scapholeberis mucronata (O.F.M.)		500					
Chydorus sphaericus (O.F.M.)		500					
COPEPODA							
Cyclops bicuspidatus Claus		75,000	25,000	47,200	540		1,000
Cyclops viridis Jurine	240						
Immature copepods		150,000	190,000	92,000	2,700	12,000	5,000
ALGAE							
Undetermined diatoms	*1,736,460*	*2,500,000*	*2,880,000*	*1,728,000*	*1,300,000*	*1,200,000*	*1,110,000*
Gyrosigma spp.	*43,411*	*50,000*	*25,000*				
Lysigonium granulatum (Ehr.)		*25,000,000*	*8,665,800*	*2,800,000*	*1,450,000*	*1,334,000*	*2,220,000*
Closterium acutum (Lyngb.)		*50,000*					
Coelastrum microporum (Nägel)		*40,000*					*22,000*
Ankistrodesmus falcatus (Corda)		*60,000*	*288,860*	*884,000*	*56,000*		
Actinastrum hantzschi Lager		*35,000*					*21,200*
Scenedesmus quadricauda (Turp.)			*28,000*	*58,000*		*22,000*	
Pediastrum duplex Meyen			1,000	5,800	5,400	2,000	
Scenedesmus dimorphus (Turp.)				*26,000*	*28,000*	800	
Synedra tenuissima Kütz						2,400	

TABLE III.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, JULY 26-28, 1929.

Italics indicate decantation collections.

Organisms	Monticello	Lake Decatur	Harristown	Illiopolis	Riverton	Springfield	Petersburg	Chandlerville
PROTOZOA								
Difflugia acuminata Ehr.	*15,000*					800		
Pandorina morum Bory		*400,000*			*187,200*	*24,000*	*25,500*	*69,600*
Platydorina caudata Kofoid		75,000			220			
Pleodorina illinoisensis Kofoid		125,000	660		880			
Codonella cratera (Leidy)		*1,600,000*	*490,000*	*966,000*	*1,248,000*	*1,470,000*	*357,000*	*70,000*
Difflugia lobostoma Leidy		*650,000*	*98,000*	*644,000*	*624,000*	*26,000*	*51,000*	1,260
Trachelomonas ensifera Daday		*220,000*		*32,200*	*62,000*	*73,500*	*12,000*	*70,000*
Trachelomonas hispida (Perty)		7,500			*30,000*			
Phacus longicaudus (Ehr.)		250					220	420
Euglena viridis Ehr.		*210,000*	*95,000*		*125,000*	*98,000*	*76,500*	*139,200*
Euglena acutissima Lemm.		750						*46,500*
Ceratium hirundinella O.F.M		8,700	650	7,100	1,320			2,520
Phacus pleuronectes (O.F.M.)		250						
Tintinnidium fluviatile Stein		*120,000*	*25,500*	*30,000*	*60,000*	*122,500*	*51,000*	*140,000*
Eudorina elegans Ehr.		*410,000*		*32,000*	*31,000*	*12,000*		*70,000*
Euglena oxyuris Schmarda		*21,000*		*32,000*	*30,000*			*11,000*
Dinobryon sertularia Ehr.		12,500	19,800	5,400				
Trachelomonas volvocina Ehr.		*30,000*	*49,000*		*62,000*	*245,000*	*76,500*	
Centropyxis aculeata Stein			600	2,160	850	400	440	
Arcella vulgaris Ehr.				4,320				
Gonium pectorale O.F.M.					220			

TABLE III—Continued.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, JULY 26-28, 1929.

Italics indicate decantation collections.

Organisms	Monti-cello	Lake Decatur	Harris-town	Iliopolis	Riverton	Spring-field	Peters-burg	Chandler-ville
ROTATORIA								
Brachionus calyciflorus Pallas	260	112,500	28,400	48,600	400			
Brachionus capsuliflorus Pallas		5,000	650	540	1,200	400	220	
Brachionus budapestinensis Daday		187,500	6,600	32,400	890	400		
Brachionus angularis Gosse		10,000	13,200	21,600		1,600	440	
Trichocerca pusilla (Jennings)		5,000	660	550				
Filinia longiseta (Ehr.)		62,500	9,900	37,800	13,200	6,000		1,260
Trichocerca gracilis (Gosse)		12,500		5,400	1,300	800	400	
Asplanchna sp.		70,000	1,980	16,200	880	400		
Polyarthra trigla Ehr		75,000	33,000	64,800	4,400	450	1,320	1,300
Keratella cochlearis (Gosse)		2,500	13,200	81,000	35,200	8,000	8,800	12,600
Synchaeta pectinata (Ehr.)		225,000	2,640	2,700	2,200	2,000	450	
Pedalia mira (Hudson)		87,500	27,000	43,200	1,760			
Brachionus patulus O.F.M.		250		270				420
Diurella stylata Eyferth		250						
Conochiloides natans (Seligo)		250		1,620				
Schizocerca diversicornis Daday								
CLADOCERA								
Diaphanosoma brachyurum (Liéven)	250	9,900	2,700					
Moina affinis Birge	500	650	540					
Daphnia longispina (O.F.M.)		660	540					
Bosmina longirostris (O.F.M.)		650						

TABLE III—Concluded.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, JULY 26-28, 1929.

Italics indicate decantation collections.

Organisms	Monti-cello	Lake Decatur	Harris-town	Illiopolis	Riverton	Spring-field	Peters-burg	Chandler-ville
COPEPODA								
Cyclops bicuspidatus Claus		5,500	5,200	5,400	880	800	880	3,800
Diaptomus siciloides Lillje		120	650	550	440			
Immature copepods	260	37,500	19,800	5,500	2,200	800	880	25,200
ALGAE								
Undetermined Diatoms	*936,000*	*5,000*	*735,000*	*322,000*	*1,560,000*	*490,000*	*25,000*	*3,480,000*
Gyrosigma sp.	*15,500*		*980,000*	1,620	450	*24,500*		
Closterium acutum (Lyngb.)	250							
Lysigonium granulatum (Ehr.)		*10,000,000*	*4,900,000*	*644,000*	*1,872,000*	*49,000*	*127,500*	*139,200*
Scenedesmus quadricauda (Turp.)		*40,000*			*31,200*	*24,000*		
Scenedesmus dimorphus (Turp.)		*20,000*	*25,500*			*23,000*	*25,500*	
Pediastrum duplex Meyen		2,500			870	1,600	440	2,520
Synedra tenuissima Kütz		12,500	*980,000*	2,160	880	2,400	880	
Pediastrum simplex Meyen		240						
Actinastrum hantzschi Lager		*20,000*	*24,000*	*32,000*	*30,000*	*24,500*	*26,000*	*70,000*
Coelastrum microporum Nägel		230						
Surirella robusta Ehr.			650					
Sphinctocystis librilis (Ehr.)					*31,000*			
Closterium moniliferum (Bory)								1,260
Aphanocapsa sp.								1,300

TABLE IV.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, SEPTEMBER 10-12, 1929.

Italics indicate decantation collections.

Organisms	Monticello	Lake Decatur	Harristown	Illiopolis	Riverton	Springfield	Petersburg	Chandlerville
PROTOZOA								
Difflugia acuminata Ehr.	250		*27,000*	1620	920	500		
Eudorina elegans Ehr.	250	220					480	
Ceratium hirundinella O.F.M.	250	880						5,600
Euglena viridis Ehr.	*62,000*	*1,222,100*	*1,310,000*	*1,400,000*	*97,600*	*1,385,000*	*400,000*	*534,000*
Pleodorina illinoisensis Kofoid		200				500		
Euglena oxyuris Schmarda		*60,000*	*30,000*	*35,000*	*24,000*		*160,000*	
Difflugia lobostoma Leidy		*288,000*	*32,000*	*12,000*	*25,000*	*28,000*	*20,000*	
Codonella cratera (Leidy)		*489,000*	*27,000*	*14,000*	*10,000*	*27,000*	*40,000*	2,800
Phacus longicaudus (Ehr.)		*24,400*	*14,000*	540	1,380	500	1,920	
Trachelomonas hispida (Perty)		440	*27,700*			*2,770,000*	*1,600,000*	
Chlamydomonas spp.		*488,840*				*6,925,000*		
Arcella vulgaris Ehr.			*28,000*	5,500	3,060	5,000		280
Centropyxis aculeata Stein			480	1,080				
Trachelomonas volvocina Ehr.			*1,668,000*	*1,750,000*	*1,220,000*	*48,475,000*	*6,400,000*	*2,670,000*
Phacus acuminata Stokes			*13,000*				*34,000*	
Trachelomonas ensifera Daday				*70,000*	*25,000*	*1,385,000*	*4,000,000*	
Phacus pleuronectes (O.F.M.)				250		500		
Euglena acutissima Lemm.				*70,000*	*20,000*		*80,000*	
Glenodinium sp.					*45,000*	*28,000*	*75,000*	
Tintinnidium fluviatile Stein					*976,000*	*21,930,000*	*4,000,000*	*26,700*
Pandorina morum Bory					*24,400*		480	
Gonium pectorale O.F.M.						*14,000*		
Platydorina caudata Kofoid							2,880	

TABLE IV—Continued.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, SEPTEMBER 10-12, 1929.

Italics indicate decantation collections.

Organisms	Monticello	Lake Decatur	Harristown	Illiopolis	Riverton	Springfield	Petersburg	Chandlerville
ROTATORIA								
Brachionus angularis Gosse	250	2,640	240				960	1,400
Brachionus budapestinensis Daday	250							560
Brachionus calyciflorus Pallas	250	850			1,840	375,000	196,800	67,200
Distylla spinifera Western	250				1,600			
Polyarthra trigla Ehr.	250	8,800	480		500	15,000	1,920	1,400
Synchaeta pectinata (Ehr.)	250				980		9,600	
Asplanchna sp.	120						950	
Keratella cochlearis (Gosse)		860	480		230			
Brachionus capsuliflorus Pallas				250	9,200	80,000	2,400	2,800
Synchaeta stylata Wierz.					13,800	1,050,000	384,000	8,400
Trichotria tetractis (Ehr.)					5,060			
Diurella stylata Eyferth						500		
Trichocerca pusilla (Jennings)						5,000		
Trichocerca gracilis (Gosse)						10,000		
Asplanchna sp.						1,000		2,100
Rotaria neptunia (Ehr.)							480	
Trichocerca stylata (Gosse)							450	
Annureopsis fissa (Gosse)								270
CLADOCERA								
Diaphanosoma brachyurum (Liéven)		450						
Daphnia longispina (O.F.M.)		4,500						

TABLE IV—Concluded.

NUMBERS OF PLANKTON ORGANISMS PER CUBIC METER IN THE SANGAMON RIVER, SEPTEMBER 10-12, 1929.

Italics indicate decantation collections.

Organisms	Monti-cello	Lake Decatur	Harris-town	Illiopolis	Riverton	Spring-field	Peters-burg	Chandler-ville
COPEPODA								
Diaptomus siciloides Lillje		6,600	4,800	600	250			
Cyclops viridis Jurine	110	450						
Immature copepods		52,800	9,600	1,200	50	500	900	2,900
ALGAE								
Undetermined diatoms	*3,120,000*	*1,222,100*	*55,550,000*	*35,000,000*	*3,660,000*	*11,080,000*	*10,000,000*	*53,400,000*
Gyrosigma spp.	*936,000*		*277,750*	*350,000*	*732,000*	*28,000*	*160,000*	*1,602,000*
Sphinctocystis librilis (Ehr.)	*15,000*		960					550
Schroederia setigera (Schröder)	*31,200*							
Cyclotella spp.		*2,444,200*				*27,700,000*		*26,700,000*
Pediastrum duplex Meyen		440	7,200	16,200	9,200	45,000	192,000	56,000
Lysigonium granulatum (Ehr.)		*3,660,300*	*22,220,000*	*17,500,000*	*244,000*	*11,080,000*	*4,000,000*	*1,335,000*
Scenedesmus quadricauda (Turp.)		2,200	*111,100*	*1,050,000*	*122,000*	*1,662,000*	*9,600,000*	*2,136,000*
Closterium acutum (Lyngb.)		*122,000*	*277,750*	*350,000*	*24,000*	*13,000*	*800,000*	*276,000*
Selenastrum gracile Reinsch		220						
Closterium acerosum (Schrank)			960				480	
Closterium moniliferum (Bory)			480					540
Ankistrodesmus falcatus (Corda)			*84,713,750*	*14,000,000*	*45,000*	*554,000*	*800,000*	*1,602,000*
Scenedesmus dimorphus (Turp.)			*55,550*	*35,000*	*46,000*	*210,000*	*800,000*	*560,000*
Synedra tenuissima Kütz			*416,625*	*700,000*	*125,000*	*1,108,000*	*70,000*	*534,000*
Coelastrum microporum Nägel				*10,000*	*12,000*	*25,000*	*75,000*	*26,000*
Cosmarium sp.				*10,800*	1,820			
Actinastrum hantzschi Lager				*700,000*	*24,000*	*12,465,000*	*3,200,000*	*1,835,000*
Micratinium pusillum							1,440	5600
Surirella robusta Ehr.					4,600			
Pediastrum simplex Meyen							450	

Reproduced With Permission From:
PUBLIC HEALTH REPORTS
54(1939) : 740-746

AQUATIC LIFE IN WATERS POLLUTED BY ACID MINE WASTE*

James B. Lackey

Cytologist, United States Public Health Service, Stream Pollution Investigations
Cincinnati, Ohio

A visitor to coal mining regions for the first time usually remarks the colored water of the streams or strip pits there. Clear red or copper colored, they are much more attractive, from an aesthetic viewpoint, than the black or milky waters produced by industrial or domestic pollution in densely populated areas.

Such copper colored waters, however, represent an extreme of industrial pollution. Coal seams contain sulfur, which, when exposed to air, oxidizes in the presence of water, and so the streams or strip pits have a very high sulfuric acid content; pH values as low as 1.8, representing 35,000 p.p.m. of acid, have been noted. Such acidities are very damaging; water works superintendents or industrial engineers needing boiler water find mine water almost useless; cattle will not drink it, and fish and most plants are quickly killed by it.

These mine runs and pits also represent an environmental extreme. Extreme environments, however, often have their inhabitants, and such is the case with the acid mine waters. One of the higher plants, the cattail, Typha latifolia, grows well in the most acid waters; and several insects, such as Chironomous, the bloodworm, caddis flies, mosquitoes, and a few beetles thrive therein. The most abundant population, however, consists of protozoa and algae, unless the bacteria, insufficiently investigated, might be more abundant.

In the past year more than 200 mine runs or pits have been personally visited and samples taken therefrom to determine their microscopic flora and fauna. The general features were noted of each location visited, the pH was determined, and, if the water was acid, a sampling station was selected which showed some pooling, if in a stream, and with an accumulation of debris in which small organisms might find lodgment. Early samples showed that suspended forms were extremely rare, and an effort was thereafter made to get those forms which might crawl or burrow into debris and bottom films. Samples taken from such situations also tended to include swimming forms, because they had been taken in still water. In April and October 1938, West Virginia mine streams and Indiana strip pits were sampled. In general, the temperatures of mine streams tend to approximate 21° C. on issuing from the mines, for mine temperatures are fairly uniform throughout the year. Strip pits, of course, tend to conform to atmospheric temperatures. Frequently, two samples were taken -- one as close to the mine mouth as possible, yet at a sufficient distance to have been seeded by surface run-off, and another from the same stream or the stream system several miles below. These two samples thus afforded opportunity to show whether animals and plants gradually invaded the stream or strip pits as acidity decreased, and also tended to show how extensive a seeding was necessary to establish life in such waters. The pH of nearby pools, streams, and swamps, not polluted by mine wastes, was determined and their flora and fauna were listed for comparison. By examining widely separated points, it was ascertained that the paucity of living species was not a local condition, but was general for acid mine waters.

Field examination of mine streams in the Spring (1) indicated abundant growths of some algae. Most usual was a green coating along banks, on debris, on rocks in the swiftest currents, and even on vertical moist rock faces. A thin brown coating was also evident at times. A heavy white growth which was common usually proved to be bacterial zooglea. Fungi were scarce, rarely forming extensive growths.

Nonbacterial microscopic organisms were composed principally of protozoa, algae, and rotifers. Table 1 shows the distribution in the plant and animal kingdoms of species found in the samples within the pH range 1.8 to 3.9. All of the commonly occurring ones were identified, but perhaps an additional 10 percent of rarely occurring species could not be recognized. Some identifications may be questionable, especially of very small forms such as the smaller chlamydomonads, which might be zoospores of Stichococcus or Ulothrix. Species definitions had to be based on rather hurried determination of morphological characters, but were usually satisfactory.

A total of 99 species of plants and animals was found living at or below pH 3.9, 85 or which were microscopic types, 76 being algae or protozoa; but the list of commonly occurring microscopic forms included only 17 species. Figure 1 shows the percentage of occurrence in all samples in which these 17 species were found. An organism was arbitrarily termed "common" if it appeared in 15 percent of the samples, "tolerant" if it appeared in 5 percent of the samples, and "adventitious" if it appeared in less than that number. This

---

* Presented at meeting of the Limnological Society of America, Richmond, Va., December 27-31, 1938.

TABLE 1.—*Distribution of recognized genera and species of plants and animals occurring at or below pH 3.9*

Plants	Number of species	Animals	Number of species
Thallophyta:		Protozoa:	
Fungi	2	Mastigophora:	
Algae:		Euglenidae	7
Myxophyceae	3	Protomastigina	7
Chrysophyceae:		Sarcodina:	
Chrysomonadales	3	Rhizopoda	15
Chrysotrichales	1	Heliozoa	2
Bacillarieae:		Infusoria:	
Pennales	5	Ciliata	19
Chlorophyceae:		Trochelminthes:	
Volvocales	1	Rotatoria	6
Ulotrichales	2	Gastrotricha	1
Chlorococcales	1	Nemathelminthes:	
Zynematales	6	Nematoda	1
Dinophyceae	2	Arthropoda:	
Bryophyta	1	Crustacea:	
Pteridophyta	1	Isopoda	1
Spermatophyta	1	Copepoda	1
		Arachnida:	
		Tardigrada	1
		Insecta	8
		Amphibia	1

Table 1 - Distribution of recognized genera and species of plants and animals occurring at or below pH 3.9.

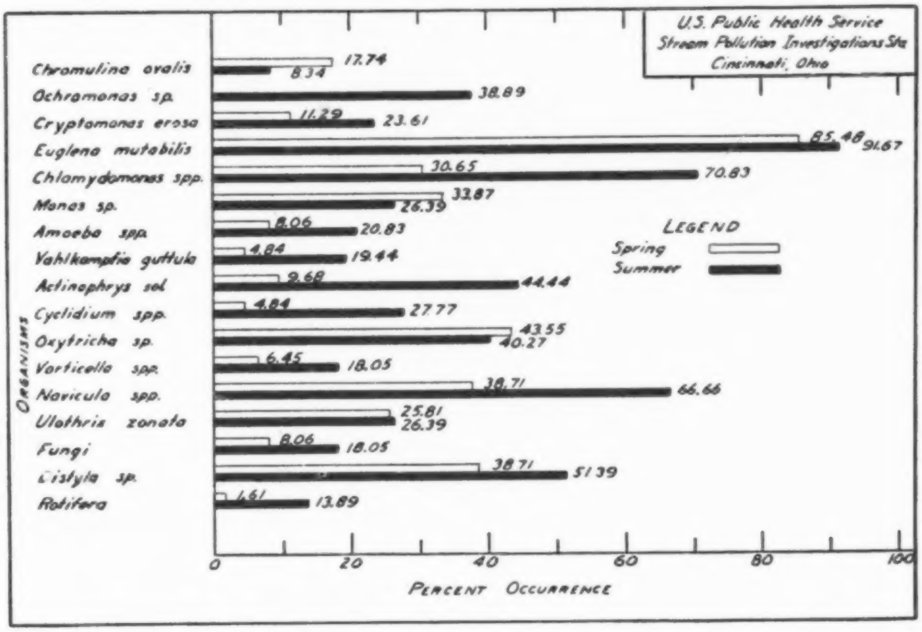


Figure 1 - Percentage of occurrence of the 17 most common organisms in all samples.

arbitrary classification is, of course, open to criticisim, but it serves as a working basis. One of its worst features is that occasionally an organism might be found in but a single sample, yet occur in such large numbers in that sample as to leave no doubt of its tolerance for that particular environmental niche. As an example of this might be mentioned the large numbers of Lepocinclis ovum which were present in Crab Orchard Creek (pH 2.5), where it was the dominant one of six species of microorganisms; or the large number of Raphidiophrys pallida in Riverdale (pH 3.0). Both of these would normally be listed as adventitious forms, but in the particular samples under consideration they were decidedly not. Amoeba radiosa is also listed as an adventitious form, but in laboratory cultures of this mine water it may attain large numbers.

Because of the seasonal differences between the first and last sampling periods (early spring and late summer, respectively), considerable differences in the flora and fauna were anticipated. Actually, very little difference was found. Ochromonas sp., common in later summer, was not found in spring, and the same is true for the small amoeba, Vahlkampfia guttula. Chromulina ovalis, common in spring, was found in 11 of the early samples and in only 6 of the later ones. Frequently, however, it was found impossible to distinguish between this creature and Ochromonas, and it seems probable that some of those listed as Chromulina in the spring samples were Ochromonas. The 17 varieties of common forms appeared in more samples in the late summer, except for Pleuromonas jaculans and Urotricha farcta. Even for the adventitious species the two sets of samples showed largely the same forms, the greatest difference being among the ciliates and rhizopods.

Nor can the species which were encountered be termed rare. Euglena mutabilis (Fig. 2) is far from common unless in an acid situation, but has been recorded by the writer (2) 11 times in 165 samples over a period of several years, while Prof. W. J. Kostir (3), of Ohio State University, has maintained a pure culture of it over a long period. Neither the Chromulina (Figs. 3, 4) nor the Ochromonas (Fig. 5) fit exactly into those species given by Pascher and Lemmermann (4), but they hardly exhibit sufficient differences to be called new species. Three of the ciliates, Chilodonella, Cinetochilum, and Glaucoma, have been shown elsewhere (2) to tolerate wide differences of environment. Probably it is just such species, i.e., those with a wide tolerance, which we might expect to find in these acid waters. The condition has been created largely by man and is, therefore, relatively recent; such

AQUATIC LIFE IN ACID WATERS

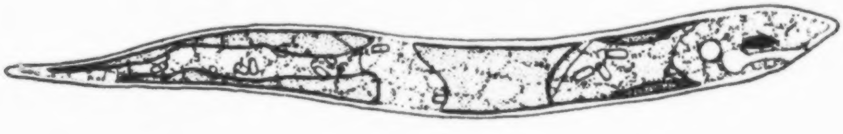
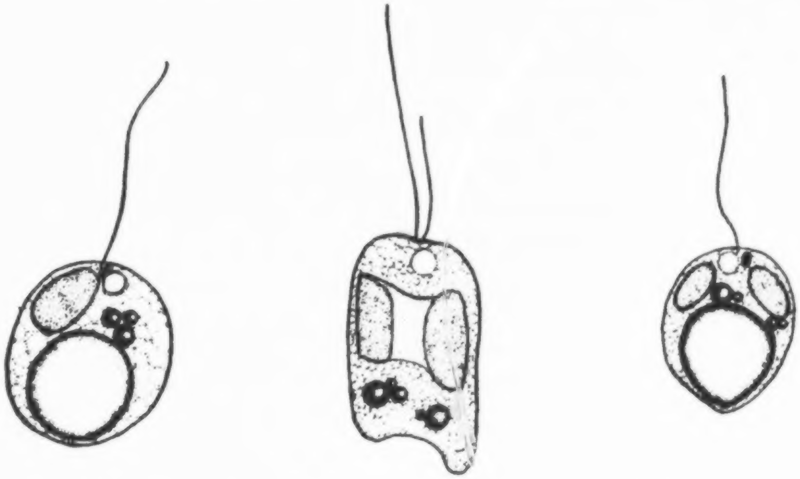


Figure 2 - Euglena mutabilis, showing two or three heavy chloroplastids, conspicuous stigma, small rod-like paramylum bodies, and apparent absence of flagellum.

like paramylum bodies, and apparent absence of flagellum.



Figures 3 and 4 - Chromulina sp., showing one or two chromatophores, stigma, and large posterior granula.

Figure 5 - Ochromonas sp., showing band-like chromatophore and absence of stigma.

species as could occupy the environment have done so, but few, if any, new ones have developed. The absence of acid-tolerant forms is marked for the desmids and shelled rhizopods of bog habitats; but we are dealing here with higher acidities than those of bogs and with a mineral acidity rather than organic acidity.

There is a very large difference between the total number of species found in one of these highly acid samples and in a sample from a stream of stagnant pool or strip pit immediately adjacent to the mine water sample, but whose pH is near neutrality. Any mine water sample could be repeatedly examined with great care day after day and never show more than a few species of microorganisms. Figure 6 shows the average number of species per sample at observed pH values up to and including 3.9. Between pH 3.9 and 4.8 very few samples were obtained; but at 4.8 and above, the number of species which could be counted increased greatly. Thus, 15 samples from pH 4.8 to 7.2, secured for comparison in the early spring trip to the mine fields, showed an average number of 23 microscopic species per sample, and the notation was made for each of these samples: "A complete list * * * not compiled * * *." Almost any 100-ml sample of Scioto River (Ohio) water, taken at the same time of year, will show from 60 to 120 plankton species alone. It is an inevitable conclusion that the highly acid waters greatly diminish the number of possible inhabitants therein.

A number of Indiana and Illinois strip pits have been dammed at various times, raising the water above the exposed coal seams and creating long and often deep and beautiful lakes. Here there is little or no chance for the oxidation of sulfur to sulfuric acid. The result is a very slow decrease in acidity and a subsequent slow repopulation of the lake by microorganisms, then by fish and other animals. The Tygart River at Phillipi, W. Va., gave a sample whose pH was 6.0 and which yielded 44 microscopic species on an incomplete examination. The river was clear and green at that point because of algae growing on submerged objects, yet a few years ago, before the sealing of mines in this region, it was a highly acid stream, "red and nothing would grow in it." No data were available on the succession of forms reinvading gradually improving streams or lakes, but copepod Crustacea were found in enormous numbers in two lakes, one with a pH of 6.6, and in the Tygart River. Because the strip pit lake is usually surrounded by high, steep banks and its total watershed area is hardly greater than the lake area, it must depend on photosynthetic protozoa and algae for fertility. The high, steep banks can contribute no humus for feeding the organisms initiating food chains, and either there are no shallow areas for growth of higher plants, or else the acid tolerant Typha preempts such areas and is, apparently, a poor "fertilizing" plant. The general impression is that recovery of a highly acid strip pit to a productive body of water is a slow process if left to nature.

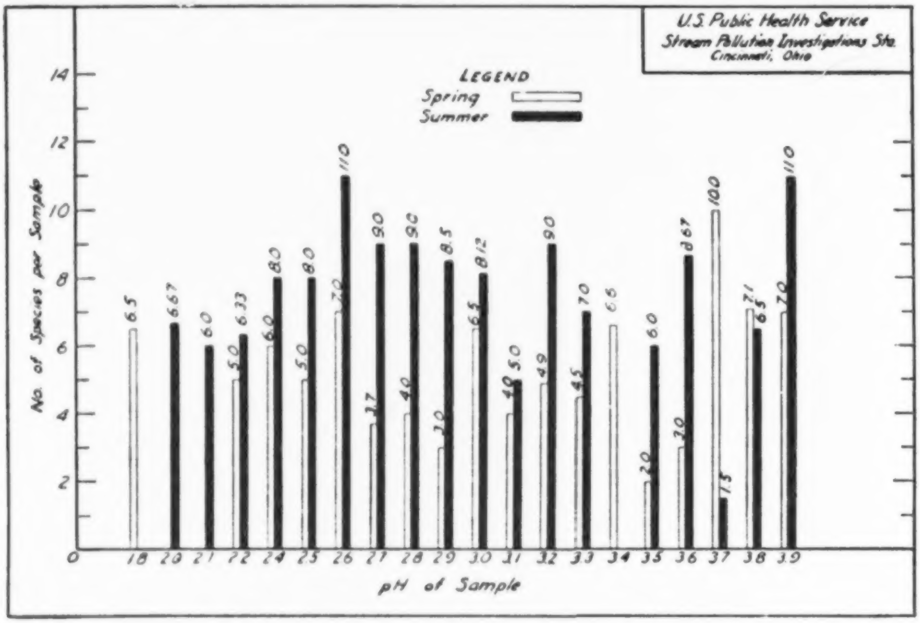


Figure 6 - Average number of species per sample within the pH range 1.8 to 3.9.

SUMMARY

Two coal mining regions, shaft mining areas in West Virginia, and strip mining areas in Indiana and Illinois, were visited and biological surveys twice made of their highly acid streams and strip pit lakes. A few adjacent almost neutral streams and lakes were surveyed for comparison.

A total of 86 species of microscopic forms was recognized. Besides Thallophyta, Protoza, and Trochelminthes, only one of the remaining phyla of plants and animals, the Arthropoda, was represented by more than one commonly occurring species in these acid waters.

At or below pH 3.9, the number of species found in any given habitat was very small. The largest number was 11 at pH 2.6 and several samples showed no life on examination.

Practically the same forms were common in April and October, but there was quite a difference in the species termed adventitious which were found at the two different times.

Seventeen species occurred in 15 percent or more of the samples and are termed "common." The most frequently occurring ones were as follows: *Euglena mutabilis*, *Naviculoid diatoms*, *Chlamydomonas* spp., *Distyla* sp., *Actinophrys sol.*, *Oxytricha* sp., *Ochromonas* sp., and *Ulothrix zonata*.

Because the most sharply definitive factor, sulfuric acid acidity, remains relatively constant, the relative constancy of species occurrence indicates that this one factor outweighs all others.

After the strip pit lakes have been sealed to reduce acid production there appears to be little chance for them to become productive except by the initial development of a large flora and fauna of chlorophyll-bearing organisms. Inasmuch as seven of the 17 organisms most common in this environment belong to this category, this initial process is apparently already under way.

REFERENCES

1. Lackey, James B.: The flora and fauna of surface waters polluted by acid mine drainage. Pub. Health Rep., 53 : 1499-1507 (August 26, 1938). Reprint No. 1976.
2. Idem: A study of some ecologic factors affecting the distribution of protozoa. Ecologic Monographs, 8 (4) : 501-527 (October 1938).
3. Kostir, W. J.: Personal communication to the writer. 1938.
4. Pascher, A., and Lemmermann, E.: Die Susswasser-flora Deutschlands, Osterreichs und der Schweiz. Gustave Fischer, Jena, 1913.

Reproduced With Permission From:
TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY
75(1945) : 175-130

A HEAVY MORTALITY OF FISHES RESULTING FROM THE DECOMPOSITION OF ALGAE IN THE YAHARA RIVER, WISCONSIN

Kenneth M. Mackenthun and Elmer F. Herman
Department of Conservation, Madison, Wisconsin

and

Alfred F. Bartsch
State Board of Health, Madison, Wisconsin

ABSTRACT

A heavy loss of fish occurred in the Yahara River below Lake Kegonsa, Wisconsin, during the latter part of September and the early part of October, 1946. All species of fish in the river were affected in the mortality. The fish, crowded close to shore, were breathing at the surface and showed marked signs of distress before expiring.

Chemical analyses of the water were made in successive periods, and experiments were performed to determine the toxicity of the river water to experimental fish. Death was attributed primarily to the depletion of the oxygen supply by the decomposing algal mass consisting of almost a pure culture of Aphanizomenon flos aquae. Secondarily, toxic substances liberated into the water by the decomposing algae probably contributed to the death of the fish.

INTRODUCTION

A heavy loss of fish occurred in that portion of the Yahara River from the Lake Kegonsa Lock downstream to its confluence with the Rock River during the latter part of September and the early part of October, 1946. Although many thousands of fish were observed, no estimate was made of the number. Carp (Cyprinus carpio) were the predominant fish affected. Other species observed were northern pike (Esox lucius), yellow pike perch or walleye (Stizostedion v. vitreum), black crappies (Pomoxis nigro-maculatus), bluegills (Lepomis macrochirus), suckers (Catostomus commersonnii), black bullheads (Ameiurus m. melas), buffalo (Ictiobus bubalus), hog suckers (Hypentelium nigricans), and an eel (Anguilla bostoniensis).

A series of eight representative stations was established in the 17 miles of river between the Lake Kegonsa Lock and the Rock River to study the progress of the mortality (Fig. 1).

PROGRESS OF THE MORTALITY

A huge algal mass estimated between three and four acres in area and several inches thick was moved into the bay above the Lake Kegonsa Lock by westerly winds and accompanying wave action on September 25, 1946. To prevent undesirable odors, the mass was permitted to pass through the lock by the lock tender over a 6-hour period. On September 28, 1946, it was reported that a heavy fish mortality was occurring at Stoughton, Wisconsin, 3-1/2 miles below the lock. Upon investigation many dead and dying fish were seen at Station 2. The fish that were still alive showed signs of acute distress and were packed close to the shore, gasping at the surface. The bay at this location provided a concentration point for fish which undoubtedly were driven out of deeper water by the oxygen deficiency caused by decomposition of the algal mass (Fig. 2). Dead fish were seen floating through the lock at Stoughton, Wisconsin, located at Station 3.

On October 1, 1946, mortality was apparent at Station 4 which is 2-1/2 miles below the previous station. Over 8,000 carp, averaging 4 pounds each, were crowded into a shallow, spring-fed stream about 500 feet long and 4 feet wide which empties into the Yahara River near this station. Above a natural fish barrier in the stream the water contained 9.6 p.p.m. of dissolved oxygen but in the lower portion there was no oxygen. The carp lived in the section of the stream where the oxygen supply remained adequate for their needs (Fig. 3).

On October 3, 1946, the mortality had reached Stations 5 and 6, located 8 miles down the river. The conditions found here were similar to those at the previous stations.

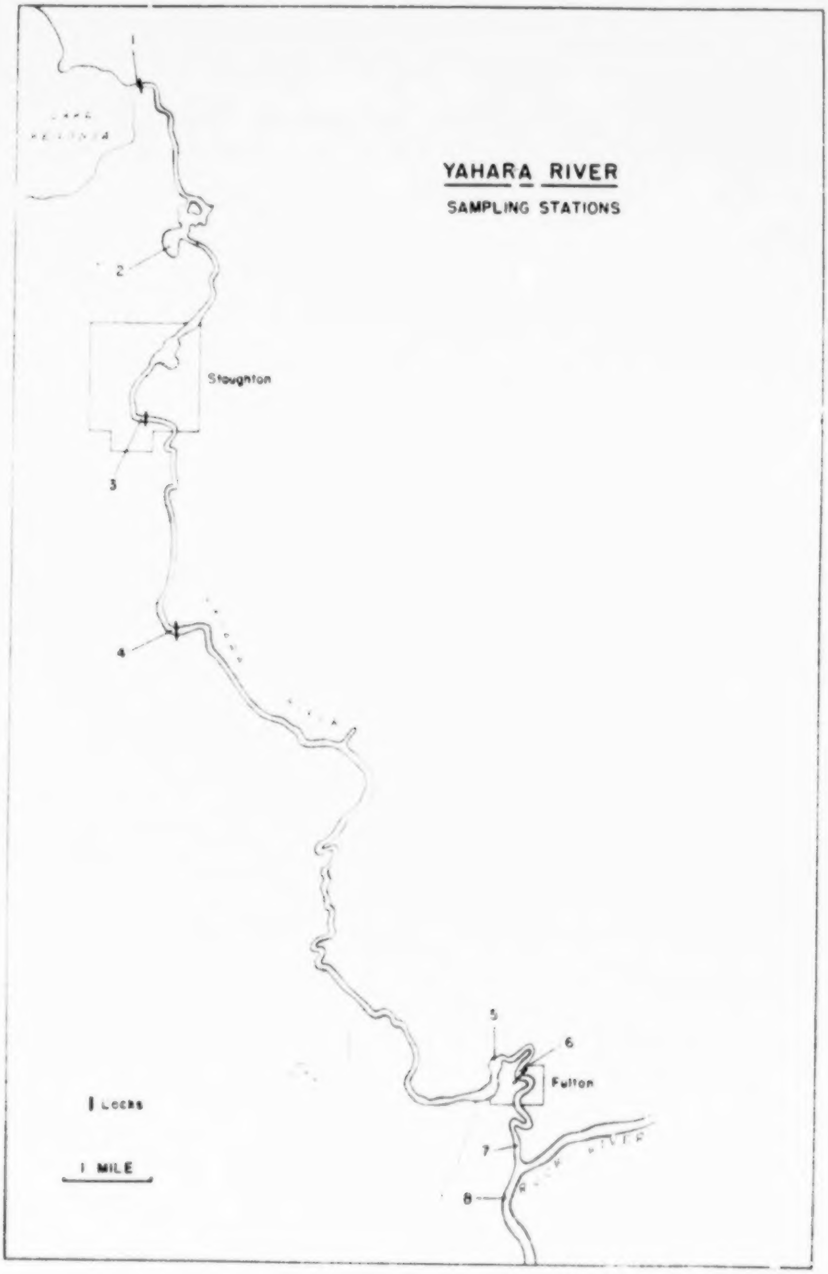


Figure 1 - Station locations on Yahara River, Wisconsin.

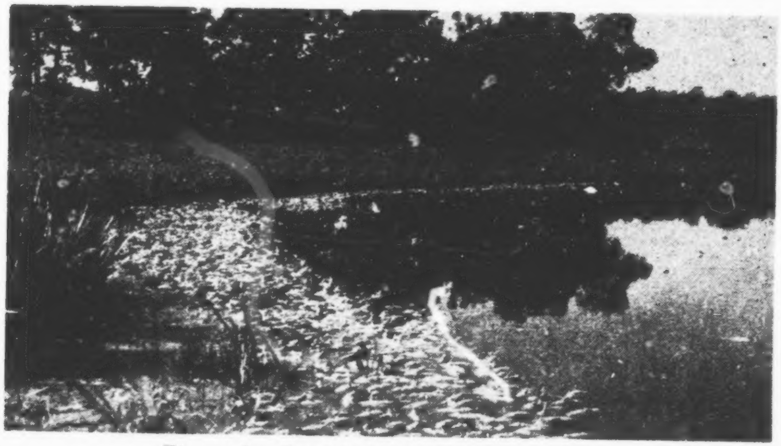


Figure 2 - Dead fish in bay of Yahara River.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Item	Date	Stations							
		1	2	3	4	5	6	7	8
Air temperature (F.°)	Sept. 28	....	58	58	....	....	....	....	....
	Oct. 1	66	65	66	....	....	....	....	....
	do. 3	....	72	72	72	72	72	72	72
	do. 5	....	68	68	68	68	....	....	....
	do. 9	....	66	66	66	66	66	....	....
Water temperature (F.°)	Sept. 28	....	62	62	....	....	....	....	....
	Oct. 1	54	64	56	....	....	....	....	....
	do. 3	....	64	58	59	60	60	60	60
	do. 5	....	62	63	61	64	....	....	....
	do. 9	....	62	60	61	61	61	....	....
Dissolved oxygen (p.p.m.)	Sept. 28	....	[illegible]	[illegible]	....	....	....	....	....
	Oct. 1	10.2	0.4	0.8	0.8	....	....	....	....
	do. 3	....	1.5	1.8	2.4	0.2	0.1	1.0	7.8
	do. 5	....	1.8	3.7	3.8	4.5	4.3	....	....
	do. 9	....	5.1	6.4	7.8	10.0	10.4	....	....
Free carbon dioxide (p.p.m.)	Sept. 28	....	43.0	30.0	....	....	....	....	....
	Oct. 1	0.0	17.5	7.0	7.0	....	....	....	....
	do. 3	....	14.0	5.7	5.5	11.5	14.4	8.0	0.0
	do. 5	....	3.5	2.0	3.0	2.0	4.0	....	....
	do. 9	....	1.0	0.0	0.0	0.0	0.0	....	....
Hydrogen-ion concentration (pH)	Sept. 28	....	7.0	7.0	....	....	....	....	....
	Oct. 1	....	7.2	7.3	....	....	....	....	....
	do. 3	....	7.3	7.7	7.4	7.4	7.3	7.4	....
	do. 5	....	7.6	7.6	7.6	7.6	7.6	....	....
	do. 9	....	7.7	8.0	8.0	8.4	8.4	....	....
Days of maximum fish mortality	Sept. 28	....	xxxx	xxxx	....	....	....	....	....
	Oct. 1	....	....	....	xxx	....	....	....	....
	do. 3	....	....	....	....	xxxx	xxxx	....	....

Table 1 - Temperature and chemical characteristics of Yahara River during the period between September 28 and October 9, 1946.

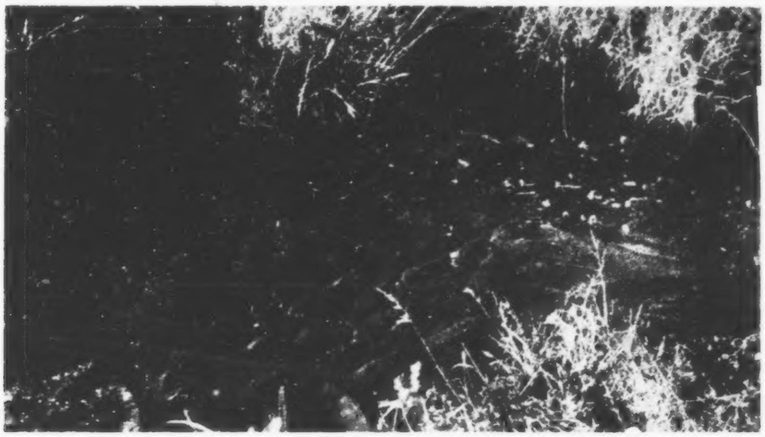


Figure 3 - Carp crowding into small spring-fed stream.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

WATER CONDITIONS

An examination of the river water at the time of the fish mortality showed a concentration of the blue-green alga, Aphanizomenon flos aquae.

The results of temperature and chemical determination are shown in Table 1. At the time of greatest mortality, the oxygen content was less than 1 p.p.m., the free carbon dioxide was high, and the pH was low. It is believed that the depletion of oxygen in the river forced the fish into the shallow bays. The greatest fish mortality seemed to take place when the decaying algal mass moved downstream. Twelve days elapsed before the water returned to a condition suitable for fish life at Stations 2 and 3. The lower stations returned to normal more rapidly because there was greater dilution of the algal mass and more wave action.

EXPERIMENTS ON TOXICITY OF RIVER WATER

An attempt was made to determine experimentally the toxicity of substances in the river water. A sample of water was taken from Station 2 during the period of greatest mortality, placed in an aquarium, and aerated. A control was set up using aerated spring water. Two yellow perch (Perca flavescens) and two black crappies (Pomoxis nigro-maculatus) were placed both in the experimental and the control aquaria. After a period of 30 hours all experimental fish had died but the control fish were still living. The oxygen in the experimental tank at the end of the same period was 8.3 p.p.m.

On October 3, 1946, a similar experiment was conducted with water taken at Station 6, 14 miles down the river. Seven yellow perch, four black crappies and one common sucker (Catostomus commersonnii) were placed both in the experimental and the control aquaria. The yellow perch began to lose their equilibrium on October 6, 1946, and began to die on October 8, 1946. All of the fish in the experimental tank were dead on October 11, 1946, but the control fish remained alive. It is believed that the fish lived longer in this than in the earlier experiment because the algal mass was dispersed and the toxic substances diluted to such an extent that they were less harmful at the lower stations along the river.

CONCLUSIONS

It is concluded from the temperature and chemical data and from the results of experiments that the primary cause of the fish mortality was the depletion of the oxygen supply brought about by decomposition of a huge mass of Aphanizomenon flos aquae. Secondarily, toxic elements released by the decomposing algae probably increased the mortality.

An examination of the literature indicates that mortality produced by the decomposition of certain blue-green algae is not a new phenomenon. Fitch et al. (1934) who reviewed the literature on the effects of algal poisoning upon domestic animals pointed out that cattle, sheep, hogs, chickens, ducks, turkeys, and geese have been known to die soon after drinking water that contained a heavy algal growth. Rabbits and guinea pigs died suddenly after being inoculated intraperitoneally with algal suspensions extracted from live algae. Prescott (1939) stated that heavy growths of phytoplankton will deplete the oxygen supply during warm still nights and that the exhaustion of the oxygen brings about the death of both microfauna and phytoplankton. The decomposition by bacteria of this mass of organic matter quickly reduces further the oxygen content. As a result, the fish and other aquatic animals are suffocated. Prescott stated further that, "it is apparently possible for algae to bring about the death of fish through the liberation of substances toxic to them during the decay process. When highly proteinaceous blue-green algae undergo decay, sufficient quantities of hydroxylamine and other derivatives are produced to poison any fish caught in the shallow water of a bay by masses of decaying algae."

LITERATURE CITED

Fitch, C. F., Lucille M. Bishop, W. L. Boyd, R. A. Gortner, C. F. Rogers, and Josephine E. Tilden. 1934. "Water Bloom" as a cause of poisoning in domestic animals. Cornell Veterinarian, Vol. 24, No. 1, pp. 30-39.

Prescott, G. W. 1939. Some relationships of phytoplankton to limnology and aquatic biology. In Problems of Lake Biology, A.A.A.S., Pub. No. 10, pp. 65-78.

Reproduced With Permission From:
SEWAGE AND INDUSTRIAL WASTES
27(1955) : 1183-1188

SUGGESTED CLASSIFICATION OF ALGAE AND PROTOZOA IN SANITARY SCIENCE

C. Mervin Palmer and William Marcus Ingram

Respectively, In charge, Interference Organism Studies; and Biologist, Water Pollution Control,
Water Supply and Water Pollution Control Research, Robert A. Taft
Sanitary Engineering Center, USPHS, Cincinnati, Ohio

Many types of microorganisms are of real importance in the field of sanitary science. Bacteria, molds, yeasts, protozoa and algae all play significant roles in relation to water and sewage. A limited number of them are pathogenic but the great majority are those that cause nuisance conditions in water supplies or are associated with sewage treatment and stream self-purification.

CLASSIFICATION OF PIGMENTED FLAGELLATES

Confusion exists in the literature of sanitary science in the classification of a considerable number of microorganisms. The confusion is especially evident in dealing with certain organisms which are on the borderline separating algae of the plant kingdom from protozoa of the animal kingdom, where one investigator may classify a particular organism with the algae and another place the same organism with the protozoa (1) (2).

The organisms most often involved in this confusion are those known as pigmented flagellates which have both the protozoan characteristic of being able to swim by means of flagella, and the algal characteristic of photosynthesis made possible by the presence of the pigment chlorophyll. Thus, they are intermediate between typical algae and typical protozoa, and it would depend upon which characteristic was emphasized as to whether they would be listed in the plant kingdom as swimming, flagellate algae, or in the animal kingdom as photosynthetic pigmented* protozoa. It is the authorities in the fields of protozoology and algology who are responsible for the existing confusion, since they have come to no agreement as to the classification of the organisms involved.

In an attempt to resolve opinions among authorities concerned with the characteristics an organism should have in order to be classified as a protozoan or as an alga, a large group name, the Protista, was proposed by Haeckel (3). Under it all protozoa and all one-celled algae were lumped together without distinction. The term "Protista" has not received general recognition and would be of little or no value to the sanitary scientist.

EXISTING CONFUSION IN SANITARY SCIENCE

A recent book, "Water Quality Criteria" (4) serves to illustrate the existing confusion in classification of microorganisms. The pigmented flagellate, Synura is listed on page 170 as an alga, and later, on page 333, as a protozoan. Lackey (5) in his paper, "Protozoan Plankton as Indicators of Pollution in a Flowing Stream," referred many organisms to the protozoa that he, in other papers, classified as algae, (1) (6). Turre (7), who has published photomicrographs of algae in water supplies, includes Volvox with the green algae, while Dinobryon and Synura are listed under protozoa. He considers the protozoa as one group of algae.

Mohr (8), in his paper on protozoa as indicators of pollution, lists the chlorophyll-bearing Euglena with the organisms Paramecium and Vorticella, which are nonpigmented protozoa. Brinley (9) lists the pigment-containing Ceratium and Peridinium as protozoa. Thomas and Grainger (2), in their book, "Bacteria," refer the pigmented flagellates Chalmydomonas, Uroglena, Synura, and Dinobryon, to a class of protozoa, the Mastigophora. Cox (10), in his book, "Laboratory Control of Water Purification," lists the following as protozoa: Dinobryon, Euglena, Uroglena, Synura, and the unrelated nonpigmented protozoan parasite Endamoeba histolytica. Hale (11), in recommending chemicals required for control of organisms, lists ten genera, including Chlamydomonas, under protozoa with only two nonpigmented genera in the list, namely, Bursaria and Endamoeba. Other pigmented flagellates, including Pandorina and Volvox which are closely related to Chlamydomonas, are placed with the algae. Hopkins (12) lists ten genera of chlorophyll-bearing organisms under protozoa in a table reproduced from Hale (13). Whipple, Fair, and Whipple (14) list some of the pigmented flagellates as protozoa and others as algae. In "Water Quality Criteria" (4), Synura, Dinobryon, and Uroglenopsis, all containing photosynthetic pigments, are listed under protozoa in a discussion of domestic water supplies along with such true nonpigmented protozoan parasites as Endamoeba histolytica and Balantidium coli. Pigmented forms such as Gymnodinium, Gonyaulax and Peridinium are listed as protozoa in a discussion of their relation to fish and shellfish mortality. Gonyaulax catenella, a pigmented form responsible for mussel poisoning, is considered only as a protozoan.

---

* "Pigmented" refers to chlorophyll and other photosynthetic pigments only.

Writers in the field of sanitary science who have placed pigmented flagellates under the algae include Kehr et al. (15), Hobbs (16), Prescott (17), Brinley and Katzin (18), Gainey and Lord (19), and Sorensen (20). Modern workers in the field of algology also follow this practice, including Fritsch (21), Smith (22), Tiffany and Britton (23), and Prescott (24).

SIGNIFICANCE OF OXYGEN-PRODUCERS

The relationship of microorganisms, including the flagellates, to oxygen is particularly significant, especially when considering their role in treatment of sewage and in stream self-purification. The amount of dissolved oxygen is one of the limiting factors in determining the speed with which microorganisms will bring about the modification of sewage in a treatment plant or of organic wastes in a stream.

It is assumed from results of research, to date, that all organisms containing chlorophyll and other related pigments are capable of carrying on the process of photosynthesis. In this process the organisms remove carbon dioxide and water from the environment and, in the presence of light, produce oxygen and carbohydrates. Much of the oxygen is released by the organism into the water, whereas the carbohydrate is retained. The photosynthetic organisms are therefore recognized as oxygen producers.

Nonphotosynthetic organisms undergo no process by which oxygen would be released into the water. These nonpigmented forms carry on the process of respiration in which the relationship of carbon dioxide and oxygen are the opposite to that in photosynthesis since available oxygen from the environment is utilized and carbon dioxide is released. The pigmented organisms also carry on respiration in addition to photosynthesis, but during the hours of daylight the latter takes place at a much higher rate. The supersaturation of natural waters with oxygen, which is frequently encountered, is usually a result of photosynthesis. In darkness, with no photosynthesis taking place, the pigmented organisms behave in a manner similar to the nonpigmented, both groups using oxygen and releasing carbon dioxide. When the sum of the effects occurring in a typical diurnal-nocturnal cycle are considered, however, the pigmented organisms are recognized as oxygen-producers and the nonpigmented ones as oxygen-consumers. If present and active in large numbers, the oxygen-producers stimulate oxidation of organic wastes by the oxygen-consuming organisms. Both oxygen-producers and oxygen-consumers, therefore, are distinct and important groups of microorganisms for the sanitary scientist to consider.

This relationship of microorganisms to oxygen is also utilized as one of the basic characteristics in their classification. Typical algae are described as oxygen-producers, and the typical molds, protozoa, yeasts, and bacteria as nonoxygen-producers or oxygen-consumers. The confusion in the classification occurs when this characteristic is not given sufficient emphasis.

A SOLUTION TO THE PROBLEM

When the oxygen and non-oxygen producers are mixed together in a classification, it is not possible to evaluate clearly their roles in water pollution problems. As has already been indicated, this type of confusion is evident in connection with the classification of the pigmented flagellates. For the sanitary scientist, it would not be difficult to overcome the confusion, because to him the oxygen-production of these organisms needs to be taken into consideration, whereas their swimming ability is of little or no importance. The sanitary scientist, therefore, should logically group the pigmented flagellates with the photosynthetic algae rather than with the nonpigmented protozoa.

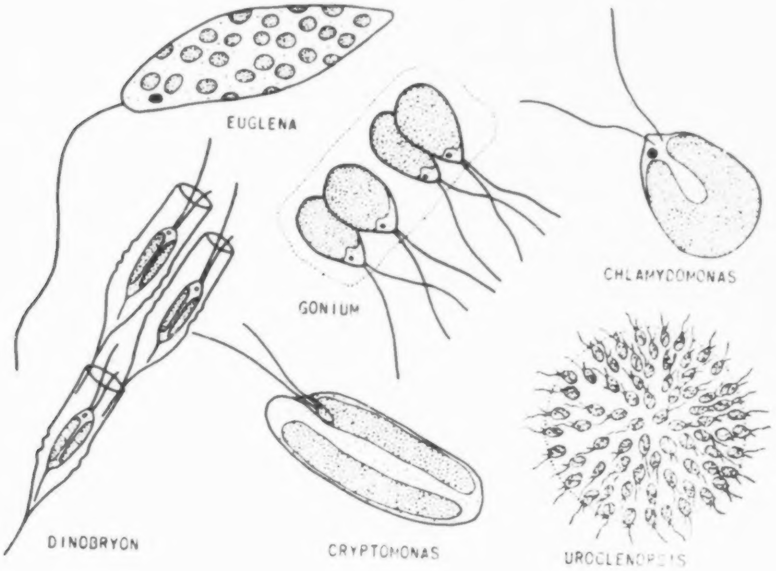
In actual practice it might be difficult or impossible to recognize oxygen-production by individual organisms were it not for the fact that the visible characteristic of pigmentation is associated with that physiological phenomenon. These organisms normally possess the green chlorophyll and frequently additional pigments in amounts sufficient to be visible under a compound microscope. The pigments are located within the protoplasm of the individual cells and can be seen to be localized within the cells of the organisms in the form of one or more plastids or chromatophores. The other pigments which may be present in addition to the chlorophyll lend various shadings of color to the plastids in the cells. The shades most frequently encountered in the pigmented flagellates are green, yellow-green, and brown. Examples of some of the pigmented flagellates with their plastids or chromatophores are illustrated in Figure 1, together with some non-pigmented flagellates. It will be noted that, in either group, both unicellular and colonial types are to be found.

In addition to the sanitary scientist's need to separate organisms according to their oxygen relationship, it is desirable from another standpoint to have all organisms grouped according to one recognized classification in order to eliminate the wasteful duplication of effort. For example, if the same swimming, pigmented organism is causing an undesirable odor in a settling basin at a water treatment installation in Iowa and at another in South Dakota, it is a handicap to have the organism listed as an alga at one place and as a protozoan at the other. Because of the presence of the photosynthetic pigment, it is recommended that all workers list it as an alga. Coming to such an agreement would do away with doubt that may arise in comparing effective control measures put into practice in each area.

NONPIGMENTED FLAGELLATES

A second small group of flagellates is involved in the confusion over classification. A few nonpigmented, swimming organisms are considered by authorities to be so closely related to the pigmented swimming forms that they are often placed in the same groups with the latter rather than with other nonpigmented forms.

PIGMENTED, OXYGEN-PRODUCING, ALGAL FLAGELLATES



NONPIGMENTED, NONOXYGEN-PRODUCING,
PROTOZOAN FLAGELLATES

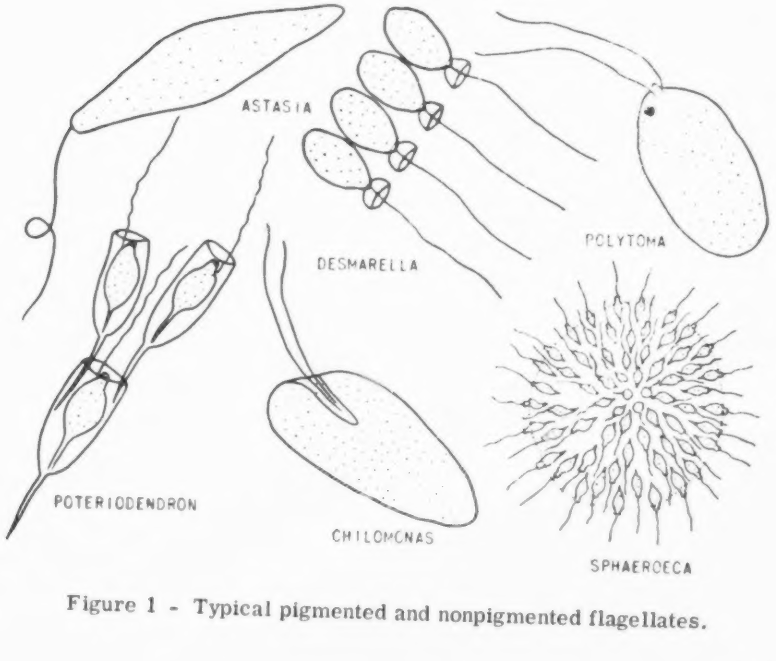


Figure 1 - Typical pigmented and nonpigmented flagellates.

Included among the nonpigmented flagellates, which have been involved in the confusion, are such genera as *Astasia*, *Polytoma*, *Chilomonas*, *Euglenopsis*, and *Peranema*. Thus, an algologist would consider these particular nonphotosynthetic organisms to be algae, just as many protozoologists have considered the pigmented flagellates to be protozoa.

Again, the sanitary scientist can settle this problem for himself by placing all nonpigmented (nonoxygen-producing) flagellates with the protozoa, disregarding the close evolutionary relationship between certain pigmented and nonpigmented forms.

RECOMMENDED GROUPING OF FLAGELLATE FORMS

Table I which follows, separating flagellates into two easily recognizable groups, can serve as a guide to classification for those working in sanitary science, and thus, make it possible to overcome the existing confusion. The first group includes the pigmented, photosynthetic, oxygen-producing, "algal" flagellates. The second group includes the nonpigmented, nonphotosynthetic, nonoxygen-producing, "protozoan" flagellates. In a very few cases, certain species of the genera in the first list are nonpigmented. These particular species would, of course, need to be placed in the second list.

More than 150 genera of flagellates have been reported for the United States. Some of these forms are comparatively rare and are of little or no importance to sanitary scientists. Others, however, are included among the microorganisms frequently encountered in water supplies or sewage. Organisms listed are limited to those flagellates which are considered to be of significance to the sanitary scientist.

SUMMARY

A lack of agreement exists among botanists and zoologists as to a definite line of demarcation between algae and protozoa. Consequently, there is no uniformity in the classification of a considerable number of flagellates which are important in the field of sanitary science. It is recommended, therefore, that the presence or absence of photosynthetic pigments (indicating the ability or inability to produce oxygen) be used in this field of applied science to separate the flagellates into the pigmented (algal) and nonpigmented (protozoan) types.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to the following who gave of their time in reading preliminary drafts of this paper: M. P. Crabill, Dr. G. W. Prescott, Dr. Kenneth E. Damann, Dr. Herbert W. Graham, M. A. Churchill, Dr. Clarence M. Tarzwell, and W. W. Towne.

TABLE I.—Classification of Flagellates

Pigmented, oxygen-producing "algal" flagellates		Nonpigmented, nonoxygen-producing "protozoan" flagellates	
Carteria	Hemidinium	Anisonema	Mastigamoeba
Ceratium	Lepocinclis	Anthophysis	Mastigella
Chlamydomonas	Mallomonas	Astasia	Menoidium
Chlorogonium	Ochromonas	Bicosoeca	Monas
Chromulina	Pandorina	Bodo	Monosiga
Chroomonas	Peridinium	Cercobodo	Noctiluca
Chrysococcus	Phacotus	Cercomonas	Notosolenus
Chrysosphaerella	Phacus	Chilomonas	Oikomonas
Colacium	Pleodorina	Clautriavia	Peranema
Cryptoglena	Pteromonas	Codonosiga	Petalomonas
Cryptomonas	Pyramimonas	Cyathomonas	Pleuromonas
Dinobryon	Pyrobotrys	Desmarella	Polytoma
Dunaliella	Rhodomonas	Dinema	Polytomella
Eudorina	Spondylomorum	Dinomonas	Poteriodendron
Euglena	Synura	Distigma	Rhabdomonas
Eutreptia	Trachelomonas	Entosiphon	Sphaeroeca
Glenodinium	Uroglena	Euglenopsis	Tetramitus
Gonium	Uroglenopsis	Heteronema	Trepomonas
Gonyaulax	Volvox	Hexamita	Tropidoscyphus
Gymnodinium	Wislouchiella	Hyalogonium	Urceolus
Haematococcus		Khawkinea	

REFERENCES

1. Lackey, J. B., "The Plankton Algae and Protozoa of Two Tennessee Rivers." American Midland Naturalist, 27, 1, 191 (1942).
2. Thomas, S., and Grainger, T. H., "Bacteria." The Blakiston Co. (1952).
3. Kudo, R. R., "Protozoology." Charles C. Thomas, Publisher, 3rd Ed. (1950).
4. Anon., "Water Quality Criteria." State Water Pollution Control Board, Sacramento, Calif. (1952).

5. Lackey, J. B., "Protozoan Plankton as Indicators of Pollution in a Flowing Stream." Pub. Health Rpts., 53, 46, 2037 (1938).

6. Lackey, J. B., Wattie, E., Kachmar, J. F., and Placak, O. R., "Some Plankton Relationships in a Small Unpolluted Stream." American Midland Naturalist, 30, 2, 403 (1943).

7. Turre, G. T., "Algae Responsible for Odor and Taste in Public Water Supplies." Proc. Amer. Soc. Civil Eng., 79, Separate 267 (1953).

8. Mohr, J. L., "Protozoa as Indicators of Pollution." Scientific Monthly, 74, 1, 7 (1952).

9. Brinley, F. J., "Plankton Population of Certain Lakes and Streams in the Rocky Mountain National Park, Colorado." Ohio Jour. of Science, 50, 5, 243 (1950).

10. Cox, C. R., "Laboratory Control of Water Purification." Case-Shepperd-Mann Publishing Corp., New York, N. Y. (1946).

11. Hale, F. E., "The Use of Copper Sulphate in Control of Microscopic Organisms." Phelps Dodge Refining Corp., New York, N.Y. (1950).

12. Hopkins, E. S., "Water Purification Control." Williams and Wilkins Co., Baltimore, Md. (1948).

13. Hale, F. E., "Controlling Microscopic Organisms in Public Water Supplies." Water Works Eng., 83, 353 (1930).

14. Whipple, G. C., Fair, G. M., and Whipple, M. C., "The Microscopy of Drinking Water." John Wiley and Sons, Inc., New York, N. Y. 4th Ed. (1948).

15. Kehr, R. W., Purdy, W. C., Lackey, J. A., Placak, O. R., and Burns, W. E., "A Study of the Pollution and Natural Purification of the Scioto River." Pub. Health Bull. No. 276, USPHS, Washington, D.C. (1941).

16. Hobbs, A. T., "Manual of British Water Supply." W. Heffer and Sons, Ltd., Cambridge, England (1950).

17. Prescott, G. W., "Objectionable Algae with Reference to the Killing of Fish and Other Animals." Hydrobiologia, 1, 1, (1948).

18. Brinley, F. J., "Distribution of Stream Plankton in the Ohio River System." American Midland Naturalist, 27, 1, 177 (1942).

19. Gainey, P. L., and Lord, T. H., "Microbiology of Water and Sewage." Prentice-Hall, Inc., New York N. Y., (1952).

20. Sorensen, I., "Biological Effects of Industrial Defilements in the River Billebergaan." Acta Limnologica (1948).

21. Fritsch, F. E., "The Structure and Reproduction of the Algae." 1, Cambridge University Press (1948).

22. Smith, G. M., "The Fresh-Water Algae of the United States." McGraw-Hill Book Co., New York, N. Y., 2nd Ed. (1950).

23. Tiffany, L. H., and Britton, M. E., "The Algae of Illinois." University of Chicago Press (1952).

24. Prescott, G. W., "Algae of the Western Great Lakes Area." Cranbrook Institute of Science (1951).

Chapter III

RELATIONSHIP TO POLLUTION OF BOTTOM ORGANISMS

Reproduced With Permission From:

INTERNATIONAL REVUE DER GESAMTEN HYDROBIOLOGIE
UND HYDROGEOGRAPHIE (INTERNATIONAL REVIEW OF HYDROBIOLOGY)
AND HYDROGEOGRAPHY) 2(1909): 126-152
TRANSLATION BY UNITED STATES JOINT PUBLICATIONS
RESEARCH SERVICE, WASHINGTON, D.C.

ECOLOGY OF ANIMAL SAPROBIA

R. Kolkwitz and M. Marsson

In the Reports of the German Botanical Society (22), we published last year the "Ecology of Plant Saprobia" which comprises about 300 species and is intended to facilitate -- as is this paper -- the evaluation of the degree of purity of streams and bodies of water.

In order to characterize the different degrees of self-purification in such waters, we distinguish three main zones and designate these by the following terms (5, 22):

I - Polysaprobiotic Zone;

II - Alpha- and Beta-mesosaprobiotic Zone;

III - Oligosaprobiotic Zone.

If we assume three adequately large and successive ponds of which the first (I) collects putrescible sewage, then the latter will have mesosaprobiotic character after passage into the second pond (II), i.e., it will be in an intermediate stage of mineralization. Upon passage into the third pond (III), the mineralization process of such water would be largely or completely terminated, i.e., the water would have an oligosaprobiotic character (15).

Zone II is asymmetrical since self-purification here takes place rather aggressively in one half of the zone and moderately in the other which explains the subdivision into alpha- and beta-mesosaprobiotic.

Similarly, we can distinguish in rivers or streams receiving putrescible substances (products of protein decomposition and carbohydrates) differing zones as far as the point at which the initial picture is reestablished.

The designation of strong and/or weak mesosaprobiotic in our plant ecological system has here been replaced by alpha- and/or beta-mesosaprobiotic because the words used can give rise to misunderstandings when taken out of context by assuming strong to mean pronounced and weak as little.

As indicated by the terms themselves, the above division into zones presupposes that the action of the chemical factors predominates over that of the physical factors unless the latter control the possibility of existence entirely, e.g. turbulent flow and entrained sand along banks and/or bottom of streams.

Division into the above zones further presuposses that putrescible organic substances exercise a considerable influence on the distribution of the organisms. Proof for the accuracy of this assumption was obtained by us from numerous investigations in many waters of a great number of different regions, especially the North-German Plain. A large part of these observations has not yet been published; however, many chemoanalytical data for this and the close parallels between biological and chemical analysis of water may be found already in our communications published in the Reports of the Institute (Royal Institute for Water Supply and Sewage Disposal). These communications concern ditches and ponds of the trickle fields at Berlin, artificial lakes in the Rhineland, the lower course of the Main River, the Elbe River from Schandau to Hamburg, the Saale River from Halle to its confluence with the Elbe and the Mleczna and Gostine Rivers in Upper Silesia to the confluence with the Vistula. There are also detailed investigations of the Elbe River at and below Hamburg in its relations to the outflow from sewers in the extensive work of Volk.

The work of Lauterborn and Marsson indicates that the Rhine has also been examined biologically but the findings of the respective chemical and bacteriological analyses have not yet been published.

The influence of the water-soluble substances on plants is generally more direct than on the animals -- no less important for the self-purification of the waters -- because the former nourish themselves by osmosis and the latter primarily by feeding on the substances. The character of the mud may therefore be of great significance for the distribution of the animals.

The dependence on the composition of the water is especially pronounced for the lower organisms. This explains the dearth in the poly- and alpha-mesosaprobiotic zone of species of higher organisms which have in greater part a less definitely graduated distribution by zone. They are therefore represented predominantly and particularly abundantly in the beta- and oligosaprobiotic zone but may overlap from here into adjacent zones through certain representatives.

An organism reacts as much more strongly to the chemical composition of water as it is more definitely saprophil -- in addition to being saprobiotic -- from the point of view of nutritional physiology which applies e.g. to Antophysa vegetans, Carchesium Lachmanni, vorticella microstoma and others. True saprophil organisms therefore are the principal guide organisms for the chemical composition of the water. The statement (August Püteer, Nutrition of Aquatic Animals, Verworn Journal for General Physiology, Vol. 7, 1907) that appreciable amounts of water-soluble organic substances also serve the nutrition of the animals would appear from the above to be valid in sweet water essentially only for certain lower organisms.

As we have stressed frequently, the main emphasis in the evaluation of the waters should in general not be laid on the individual organisms but on the biocenoses ("Bioconosen") whose particularities cannot here be described in detail. In this respect, we may well apply here the maxim coined by the systematicians according to which one (a single) character is no character. Only in tests of drinking water is it possible for isolated organisms to play an essential role as is frequently shown in the pertinent literature.

The clearest picture of the state of the water will obviously be obtained by a planned consideration of the three typical regions of open water, bank and bottom, especially if we take adequately into account the interaction of the plants and animals in them and the essentially opposite products and requirements of the latter (cf. 17 on methods and instruments for procuring samples).

For an understanding of the relations between the aquatic regions and the saprobiotic zones, we should like to stress here that, for example, a lake deriving oligosaprobiotic character from its plankton and benthos may well contain mesosaprobiotic mud organisms and often does contain them. The same lake may also change its biological picture with the seasons because, e.g., an abundant growth of hydrophyll plants dies off and thus brings mesosaprobiotic elements into the true plankton zone, especially near the banks.

The foundations of the biological evaluation of water find their basis in the proper appreciation of these combinations. Nearly always we must be concerned not only with testing the water but with determining the entire state of a collector.

In the present communication, we have placed the main emphasis on the system and restricted discussion in the text as much as possible. In a more extensive report to be published in the Reports of the Royal Institute for Water Supply and Sewage Disposal, we intend to combine the ecological systems of both plants and animal organisms.

The following brief considerations are intended to serve as a characterization in biological and chemical respect of the principal zones referred to initially.

I - The zone of polysaprobia is characterized chemically by a certain degree of wealth of high-molecular, putrescible and organic substances (protein components and carbohydrates) which enter the collectors in the directly putrescible sewage from cities and agricultural, industrial and other enterprises. A decrease of oxygen content of the water accompanied by reduction manifestations, formation of hydrogen sulphide in the mud and an increase of carbon dioxide often are the chemical sequels of this.

Organisms generally occur in great numbers but with a certain monotony; especially Schizomycetes and (usually bacteriophage) colorless flagellates are frequent. The bacteria developed in standard nutrient gelatine may exceed 1 million per ccm water. Organisms with high oxygen requirements obviously are generally completely absent. Fishes usually avoid remaining in this zone for any length of time.

Plant organisms preferring hydrogen sulphide in this zone may recur in H_2S sources in the oligosaprobiotic zone.

II - The zone of the mesosaprobia is divided into a alpha- and/or beta-saprobiotic section. It generally succeeds the polysaprobiotic zone. In the alpha-section which adjoins the former, self-purification takes place still rather agressively as already mentioned but with the simultaneous occurrence of oxidation manifestations -- in contrast to zone I -- which are conditioned in part by the oxygen production from chlorophyllous plants.

The protein components contained in the water are probably already decomposed down to asparagin, leucine, glycocoll, etc., which results in a qualitative difference from zone I.

In the beta-section, the decomposition products already approach mineralization. Normal, generally nitrate-containing effluents from the trickle fields are most properly included in this zone.

All organisms of the mesosaprobiotic zone usually are resistant to minor action by sewage and its decomposition products. Notable is among other factors the content of the zone of Diatomaceae, Schizophyceae and many Chlorophyceae and some higher plant organisms. Higher and lower animal organisms are also found in a great number of individuals and varieties.

III - The zone of the oligosaprobia is the domain of (practically) pure water. If it was preceded by a self-purification process locally or chronologically, it succeeds the mesosaprobiotic zone and then represents the termination of mineralization. However, we here include also lakes in which the water does not undergo a mineralization process properly speaking. The oxygen content of the water may often remain permanently close to the saturation limit and occasionally even exceed the latter (as a function of the air dissolved in the water). The content of organic nitrogen usually does not exceed 1 mg/lit. The water is generally transparent to a considerable depth, except at times of abundant plant growth. The number of bacteria developed on standard nutrient gelatine is generally low. In contrast to the polysaprobiotic zone, it amounts to only a few hundreds and infrequently thousands of bacteria per ccm water.

Both the plant and animal plankton of our clean country lakes belongs in this zone. We already pointed out that the mud of such waters may have a beta-mesosaprobiotic character.

Further characteristics of the three principal zones will be found in "Ecology of Plant Saprobia".

Catharobia, i.e., inhabitants of perfectly pure water, have here not been listed intentionally because they have nothing or almost nothing to do with the self-purification of any of these waters. We might count inhabitants of pure mountain streams among them (e.g. Planaria alpina Dana) but the aeration and coolness of the water plays a greater role for them on the basis of our present experience than the particular pure quality of the water.

We are probably justified in already pointing out here that the greater part of the animal organisms living in these waters can advance further into contaminated zones than we have been inclined to assume so far, provided they find the required amount of oxygen.

Ecological System of Animal Saprobia

Within the three principal ecological groups, the organisms listed here and which number more than 500, are arranged in accordance with the natural system, except for the fishes. As for the plant saprobia, we have here also based ourselves in the classification of the various animals on our own observations in the open as far as possible. We endeavored to find the centers of optimum growth and development of the organisms especially at locations with graduated self-purification and determined the position in the ecological system on the basis of these observations.

We do not want to fail to point out here that many pertinent data in literature are incomplete. This is due to the fact that until the present time too few different locations had been investigated and that there usually did not exist any chemical analysis which is highly desirable for these purposes. Occasionally an observer unfamiliar with local conditions has great difficulty in arriving at an accurate evaluation of the character of the particular body of water.

Since it was obviously impossible to enumerate all organisms occurring in water and suitable for the present purposes, we have selected those -- by avoiding any overloading with names of the system here as also in the case of the plants -- which are of definite interest for the evaluation of the state and the self-purification of water on the basis of the present investigations. We need not specifically point out that further investigations may result in future additions to the listing.

In spite of this, the present system already forms a relatively complete whole because we have listed sufficient organisms for each zone so that they already afford a thorough characterization.

In looking over the system, it will be noted that the names of Linné and Ehrenberg occur frequently which is a sign that we have utilized for evaluation as far as possible the more frequent and generally known organisms.

We have endeavored to furnish the most complete enumeration of poly- and alpha-mesosaprobiotic organisms because these are the most important (as inhabitants of the locations of intensive self-purification) for the evaluation of the water.

In order to prevent any too severe evaluations of the degree of purity of the water, we have restricted -- while trying to maintain completeness as far as possible -- the groups of the organisms just named as far as possible and have instead pointed out any possible overlapping of the purer zones into the preceding zones.

The colorless flagellates have been taken into account fully in accordance with their frequency and extensive distribution. They are particularly characteristic for the poly- and alpha-mesosaprobiotic zones.

The Chrysomonodales, Cryptomonodales, Euglenales, Peridiniales and (partly) Protococcales, all part of the flagellates, have already been treated in the plant ecology, not because we meant to stress their belonging to the vegetable kingdom but because all oxygen-producing (aerating) organisms should be grouped in one system as far as possible.

The Ciliata have the greatest significance in the evaluation of the degree of contamination of many water courses and especially their contaminating affluents which daily observation has confirmed for us over many years. They have therefore been taken into account particularly, the more so since they can be determined more easily than the small flagellates. Spongiae are in general not very suitable for evaluation of water on the basis of our present investigations although they may be often considerably advanced in their development through nutrient affluents.

Among the Vermes, the limicolus tubificids are of considerable importance for the evaluation of the poly- and alpha-mesosaprobiotic zone whereas others belong more to the zone of pure water. The nematodes, occupying the intermediary position in comparison with them, are important for microscopic analysis. The greater part of the species is of lesser importance for water evaluation on the basis of the present investigations but may play an important role in the consumption and loosening up of mud.

We gathered extensive data on the Rotatoria so that we were able to utilize them sufficiently thoroughly especially in the evaluation of streams in addition to other animal and plant groups.

Although the Bryozoa are widely distributed, they have so far been investigated very little in respect to their suitability for water evaluation.

The greater part of the molluscs have been classified in the group of oligosaprobiotic organisms. This indicates that -- similar to the higher aquatic plants -- most of them are not used very much for the differentiation of the different zones of importance in the characterization of self-purification. However, they are of the greatest importance for the evaluation of the state of water merely by their role as indicators. They may indicate toxic agents by their sudden decay, asphyxiation from lack of oxygen through putrefaction, lack of calcium through their almost complete absence, etc.

Molluscs may also play an important role as scavengers of detritus -- as do the snails as omnivora and vegetarians -- in keeping streams and other bodies of water clean.

As far as possible, Crustaceae have been considered for water investigations by us and utilized in the ecological system. However, they would seem to merit -- especially the Cyclopida -- further study in regard to the interrelation between distribution and water character.

Arachnoidea need be considered for general biological analysis only to a minor degree on the basis of our present investigations. Insect larvae and, in part, the adult insects -- with the exception of the red larvae of Chironomus and others -- play a role in the evaluation of waters only insofar as they do not advance into contaminated zones. When they occur abundantly, as is often the case, their activity in feeding and the many adult insects in their escape from the water have great importance for keeping the latter clean and may also be of considerable value as fish feed by reason of their relative size.

The various species of insect larvae could be taken into consideration only very little in the present state of science because determination of type from the larvae is usually not possible. It becomes necessary to breed the imagoes which has not yet been done on a planned basis for the present purpose either by us or anyone else to judge from the available literature.

At a summary review, most Pisces can be classified under two groups different by nature: the mud fishes often living in purposely fertilized ponds, and the predatory fish. On this basis and in consideration of other particularities of habitat, we have placed part of the fishes in the beta-mesosaprobiotic and the others in the oligosaprobiotic zone. Many fishes, in particular those with greater vital tenacity, also enter the alpha-mesosaprobiotic zone in feeding and come close to the polysaprobiotic zone but seem to prefer the purer zones wherever possible. This would seem to be valid also for their spawn.

Among the Aves, the gulls (especially Larus ridibundus L), crows (especially Corvus cornix L) and ducks (usually Anas boschas L) are noteworthy as scavengers of lumps of sewage and proliferating sewage fungi.

I. Polysaprobia

Rhizopoda

Hyalodiscus limax (Duj.) both often together with
" guttula (Duj.) Polytoma and Spirillae;
when isolated, also mesosaprobiotic.

Flagellata

Cercobodo longicauda (Duj.) Senn.
Cercomonas longicauda Duj.
Dimorpha longicauda (Duj.) Klebs.
Dimastigamoeba longicauda Klebs.,
overlaps into alpha mesosaprobiotic zone.
Oicomonas mutabilis Kent.
Bodo putrinus (Stokes) Lemm.
Trepomonas rotans Klebs., tends also to be alpha-
Hexamitus inflatus Duj. mesosaprobiotic.
" crassus Klebs.
" pusillus Klebs., overlaps into alpha-
" fissus Klebs. mesosaprobiotic zone.
" fusiformis Klebs.

Ciliata

Paramaecium putrinum Cl. & L.
Vorticella microstoma Ehrbg.
" putrina O.F. Müller

Vermes

Tubifex tubifex (O.F. Müller), when predominant and abundant.

Diptera

Eristalis tenax L., larvae, often in highly contaminated trickle-filled ditches and strongly hydrogen-sulphide containing storage areas; also in the mesosaprobiotic zone.

II. Mesosaprobia

1. Alpha-mesosaprobiotic

Rhizopoda

Trinema enchelys (Ehrbg.) Leidy.
Diplophrys archeri Barker.
Pamphagus hyalinus Leidy.
" armatus Lauterb.
Cryptodifflugia oviformis Penard.

Flagellata

Ciliophrys infusionum Cienk; frequent inhabitant of contaminated aquaria.
Cercobodo radiatus (Klebs.) Lemn.
Dimorpha radiata Klebs.
Cercomonas clavata Perty.
" crassicauda Duj.
Oicomona termo (Ehrbg.) Kent.
Monas vivipara Ehrbg.
" vulgaris (Cienk.) Senn. Monas guttula Ehrbg.
" arhabdomonas (Fisch) H. Meyer.
Anthophysa vegetans (O.F. Müll.) Bütschli., very typical for alpha mesosaprobiotic zone; when colonies die off in pure water, the stems remain.
Amphimonas globosa, Kent
" fusiformis Mez.
Bodo globosus Stein.
" mutabilis Klebs.
" minimus Klebs.
" caudatus (Duj.) Stein.
" saltans Ehrbg., also in the polysaprobiotic zone.
" ovatus (Duj.) Stein
Spongomonas intestinum (Cienk.) Kent., also in the beta-mesosaprobiotic zone.
Dallingeria drysdali Kent, also in the beta-mesosaprobiotic zone.
Pleuromonas jaculans Perty.

Phyllomitus amylophagus Klebs.
Rhynchomonas nasuta (Stokes) Klebs.
Tetramitus descissus Perty
" salcatus Klebs.
" pyriformis Klebs.
" rostratus Perty.
Urophagus rostratus (St.) Klebs.
Trigonomonas compressa Klebs.
Trepomonas agilis Duj., also in the beta-mesosaprobiotic zone.
" steini Klebs.
Menoidium pellucidum Perty.
Astasiopsis distorta (Duj.)
Astasia margaritifera Schmarda Astasiodes.
Euglenopsis vorax Klebs.
Peranema trichophorum (Ehrbg.) St.
Heteronema tremulum Zach.
" (Zygoselmis) acus (Ehrbg.) St.
Scytomonas pusilla Stein.
Chilomonas paramaecium Ehrbg., also in the beta-mesosaprobiotic zone.
Spirochaete plicatilis Ehrbg.

Ciliata

Urotricha farcta (Ehrbg.) Cl. & L.
Amphileptus claparedi Stein, possibly also polysaprobiotic.
" carchesii Stein.
Lionotus varsaviensis Wrz.
Loxophyllum meleagris (O.F. Müll.) Duj., also in the beta-mesosaprobiotic zone.
Cyclogramma rubens Perty (Nassula Clap. & L. & A.)
Chilodon uncinatus Ehrbg., also in the beta-mesosaprobiotic zone.
Trochilia palustris Stein, also in the beta-mesosaprobiotic zone.
Leucophrydium patrinum Roux.
Glaucoma scintillans Ehrbg., also in the beta-mesosaprobiotic zone.
Colpidium colpoda Stein.
Colpoda cucullus Ehrbg.
" parvifrons Cl. & L.
" steini Maupas
also in the beta-mesosaprobiotic zone.
Loxocephalus granulosus Kent.
Paramaecium caudatum Ehrbg.
Cyclidium glaucoma Ehrbg.
Spirostomum ambiguum Ehrbg., also in the beta-mesosaprobiotic zone.
Stentor coeruleus Ehrbg.
" roeseli Ehrbg., also in the beta-mesosaprobiotic zone.
Gyrocoris oxyura Stein
Caenomorpha medusula Perty, was also found abundantly in stagnant hydrogen sulphide-containing river water in which oxygen could not be demonstrated with the Winkler method.
Urostyla weissei St. U. multipes (Cl. & L.), possibly also polysaprobiotic.
Gastrostyla mystacea (St.)
Oxytricha fallax Stein.
" pellionella Ehrbg.
Stylonychia mytilus Ehrbg., also in the beta-mesosaprobiotic zone.
Gerda glans Lachm.
Vorticella convallaria Ehrbg.
Carchesium lachmanni Kent.
Epistylis coarctata, Cl. & L.
" plicatilis Ehrbg., also in the beta-mesosaprobiotic zone.

Suctoria

Podophrya carchesii Cl. & Lachm.
" fixa Ehrbg., also in the beta-mesosaprobiotic zone.

Vermes

Enchytraeus humicultor Vejd.
Pachydrilus pagenstecheri (Ratz.) Vejd.
Lumbriculus variegatus (Müll.) also in the beta-mesosaprobiotic zone.
Limnodrilus udekemianus Clap.
" hoffmeisteri Clap.
Tubifex tubifex (Müll.), overlaps into the polysaprobiotic zone (cf. the latter) and beta-mesoprobiotic zone.
Lumbricillus lineatus (Müll.), advances from the sea to contaminated brackish and/or sweet water.
Psammoryctes barbatus Vejd.
Dero limosa Leidy.
Aeolosoma quaternarium Ehrbg.
Lumbricus rubellus Hoffm.
Monohystera macrura De Man, also in the beta-mesosaprobiotic zone.
Tripyla setifera Butschli
Trilobus gracilis Bast.
Plectus tenuis Bast.
Diplogaster rivalis (Leyd.).

Rotatoria

Rotifer vulgaris Schrank, also in the beta-mesosaprobiotic zone.
" actinurus Ehrbg., occasionally polysaprobiotic; occurs in water enriched with hydrogen sulphide and poor in oxygen as demonstrated by the Winkler method.
Callidina elegans Ehrbg., and other varieties.
Triarthra longiseta Ehrbg., often abundant in contaminated village ponds and collectors which receive sewer outflow.
cf. var limnetica.
Hydatina senta Ehrbg., occurs isolated also in the weak mesosaprobiotic zone.
Diglena biraphis Gosse
" caudata Ehrbg.
also in the beta-mesosaprobiotic zone.
Diplax compressa Gosse.
" trigona Gosse.
Diplois daviesae Gosse.
Colurus bicuspidatus Ehrbg: isolated, also in the beta-mesosaprobiotic zone.
Brachionus angularis Gosse, also in the beta-mesosaprobiotic zone.
" militaris Ehrbg., also in the beta-mesosaprobiotic zone.

Mollusca

Sphaerium (Cyclas) corneum L., occurs abundantly in the Spree River downstream from the Berlin emergency outlets; very resistant to organic sewage; also in beta-mesosaprobiotic mud.

Crustacea

Asellus aquaticus (L.) Ol., when in large amounts and well developed; often between putrefying Spherotilus on which it may feed.

Neuroptera

Sialis lutaria L., larvae, very resistant, often in very much dirt; also in mud of Lake Lucerne and elsewhere.

Hemiptera

Velia currens Febr. Very resistant against contamination.

Diptera

Chironomus plumosus L., larvae, abundant occurence very typical for this region; also in the poly- and beta-mesosaprobiotic zone. This species with its red larvae is a collective species.
" motitator (L.), also in the beta-mesosaprobiotic zone.
Tanypus monilis (L.), also in the beta-mesosaprobiotic zone.
Caenia fumosa Stenh., larvae, imago along the edges of purine ditches.
Ptychoptera contaminata L., larvae; often associated with Beggiatoa and Euglena virdis.
Psychoda phalaenoides (L.), larvae
" sexpunctata Curtis.
" Ps. phalaenoides Meigen, larvae.
Stratiomys chamaeleon L., larvae.

2. Beta-mesosaprobiotic

Rhizopoda

Amoeba brachiata Duj.
" verrucosa Ehrbg.
" radiosa Ehrbg. Dactylosphaerium.
Pelomyxa palustris Greff. also alpha-mesosaprobiotic.
Cochliopodium bilimbosum Leidy.
" pellucidum (Arch.) Hertw. & Less., also alpha-mesosaprobiotic.
Arcella vulgaris Ehrbg., also alpha-mesosaprobiotic.
Centrophyxis aculeata (Ehrbg.) St.
Euglypha alveolata Duj.
Platoum stercoreum (Cienk.)
Pamphagus mutabilis Bailey.

Heliozoa

Actinophrys sol Ehrbg., also alpha-mesosaprobiotic.
Actinosphaerium eichhorni (Ehrbg.) also alpha-mesosaprobiotic.
Spaerastrum fockei (Arch.).
Clathrulina elegans Cienk., also alpha-mesosaprobiotic.

Flagellata

Mastigamoeba aspera F.E. Sch.
" invertens Klebs.
" limax Moroff.
" polyvacuolata Moroff.
Eucomonas soc[illegible]lis Moroff.
Spaeroeca volvox Lauterborn.
Bodo celer Klebs.
" rostratus (Kent) Klebs.
" uncinatus (Kent) Klebs.
" repens Klebs.
Pleuromonas jaculans Perty.
Menoidium falcatum Zach.
Phialonema cyclostomum St. = Urceolus cyclostomus (St.) Mereschk.
Anisonema acinus Duj.
Entosiphon sulcatum (Duj.) St.
Chilomonas paramaecium Ehrbg.; also alpha-mesosaprobiotic.

Ciliata

Urotricha lagenula (Ehrbg.).
Enchelys pupa Ehrbg.
" silesiaca Mez.
Prorodon farctus (Cl. & L.).
" platyodon Blochm.
Lagynus elegans (Engelm.) tends to be also oligosaprobiotic.
Coleps hirtus Ehrbg., also alpha-mesosaprobiotic.
Didinium nasutum Stein.
Disematostoma buetschlii Lauterb.
Loxophyllum armatum Cl. & L.
" (Lionotus) fasciola Cl. & L.; also alpha-mesosaprobiotic.
" lamella Cl. & L.
Trachelophyllum lamella (L. F. M.).
" pusillum Clap.
Trachelius ovuum Ehrbg.
Loxodes rostrum Ehrbg.
Nassula elegans Ehrbg.
" ornata Ehrbg.
Chilodon cucullulus Ehrbg., also alpha-mesosaprobiotic.
Opisthodon niemeccensis Stein.
Dysteropsis minuta Roux.
Frontonia acuminata (Ehrbg.) Cl. & L.
Chasmatostoma-reniforme Engelm.
Uronema griseolum (Mps.).
" marinum Duj.
Cinetochilum margaritaceum Perty.
Paramaecium bursaria (Ehrbg.) Focke.
" aurelia (O.F. Müll.) also alpha-mesosaprobiotic.
Urocentrum turbo Ehrbg.
Lembadion bullinum (O.F. Müll.) Perty.
Pleuronema chrysalis (Ehrbg.) Stein.
Balantiophorus minutus Schew., tends to be also oligosaprobiotic.
Blepharisma lateritium (Ehrbg.) Stein.
Metopus sigmoides (O.F.M.) Cl. & L., also alpha-mesosaprobiotic.

" contortus Levander.
" pyriformis Levander.
Plagiopyla nasuta Stein.
Spirostomum teres Cl. & L.
Condylostoma vorticella Ehrbg., tends to be also oligosaprobiotic.
Bursaria truncatella O.F. Müll.
Tylacidium truncatum Schew.
Climacostomum virens Stein.
Stentor polymorphus Ehrbg., tends to be also oligosaprobiotic.
" igneus Ehrbg.
" niger Ehrbg.
Halteria grandinella (O. F. Müll.).
Tintinnidium fluviatile (St.); tends to be also oligosaprobiotic.
Uroleptus musculus Ehrbg.
" piscis (Ehrbg.)
Stylonychia mytilus Ehrbg.
Euplotes patella Ehrbg.
" charon Ehrbg.
Aspidisca costata Stein
" lynceus Ehrbg.
} also alpha-mesosaprobiotic.
Astylozoon fallax Engelm.
Vorticella campanula Ehrbg.
" patellina Ehrbg.
" citrina Ehrbg.
Carchesium epistylis Cl.
Zoothamnium arbuscula Ehrbg.
Epistylis umbellaria Lachm. and other species.
Cothurnia crystallina Ehrbg.

Suctoria

Sphaerophrya pusilla Cl. & L. and other species which are in part not yet accurately determined.
Podophrya quadripartita Cl. & L.
Acineta grandis Kent.

Spongiae

Ephydatia muelleri (Lieberkuhn)
" fluviatilis (L.)
Euspongilla lacustris (L.)
Spongilla fragilis Leidy
} also overlap into adjacent zones; very little suitable for water evaluation as far as is known now.

Hydroidea

Hydrae on Lemnae occasionally also in this region; cf. oligosaprobiotic zone.

Vermes

Rhynchelmis limosella Hoffm.; isolated; also alpha-mesosaprobiotic.
Eiseniella tetraedra (Sav.). amphibian
Criodrilus lacuum Hoffm.
Nephelis vulgaris Moq.-Tand, overlaps
Clepsine bioculata (Bergm.)
" sexoculata (Bergm.)
} tends to be also oligosaprobiotic.
Nāis elinguis Müll., also alpha-mesosaprobiotic.
Stylaria lacustris (L.)
Haemopis sanguisuga (Bergm.) = Aulostomum gulo Moq.-Tand.
Polycelis nigra (Müll.) Ehrbg.
Dendrocoelum lacteum Oerst.
Cercariae with forked rudder tail (in plankton).

Rotatoria and Gastrotricha

Floscularia atrochoides Wierz.
Atrochus tentaculatus Wierz.
Melicerta ringens Schrank.
Conochilus unicornis Rousselet.
Philodina roseola Ehrbg., also alpha-mesosaprobiotic.
" erythrophthalma Ehrbg.
" megalotrocha Ehrbg.
Rotifer tardus Ehrbg.
" bulgaris Schrank, vgl., also alpha-mesosaprobiotic.
" macrurus Ehrbg.
Asplanchna priodonta Gosse, tends to be also oligosaprobiotic.
Synchaeta tremula Ehrbg., tends to be also oligosaprobiotic.
" pectinata Ehrbg. tends to be also oligosaprobiotic.
Polyarthra platyptera Ehrbg. tends to be also oligosaprobiotic.
Triarthra longiseta var limnetica (Zach) = Tr. thranites Skor.
" mystacina Ehrbg.
Taphrocampa selenura Gosse.
Proales tigridia Gosse.
Furcularia gracilis Ehrbg., also alpha-mesosaprobiotic.
" forficula Ehrbg.
" gibba Ehrbg.
" reinhardti Ehrbg., occasionally associated with Stentor coeruleus.
Diglena catellina Ehrbg., also alpha-mesosaprobiotic.
" forcipata Ehrbg., "
Dinocharis pocillum Ehrbg., tends to be also oligosaprobiotic.
" tetractis Ehrbg., "
Scaridium longicaudum Ehrbg.
Stephanops unisetatus Collins.
Diaschiza semiaperta Gosse, also alpha-mesosaprobiotic.
" tenuior Gosse.
Salpina macracantha Gosse.
" mucronata Ehrbg., also alpha-mesosaprobiotic.
Euchlanis triquetra Ehrbg.
Cathypna luna Ehrbg.
Monostyla lunaris Ehrbg.
Lepadella ovalis Ehrbg. also alpha-mesosaprobiotic.
Pterodina patina Ehrbg.,
Pompholyx sulcata Hudson.
Noteus quadricornis Ehrbg.
Brachionus militaris Ehrbg., also alpha meso-saprobiotic.
" pala Ehrbg. = B. pala-amphiceros Plate
" urceolaris Ehrbg.
" rubens Ehrbg.
" bakeri Ehrbg.
" angularis Gosse, also alpha meso-saprobiotic.
Anuraea aculeata Ehrbg.
" cochlearis Gosse
} tends also to be oligosaprobiotic.
Notholca striata Ehrbg.
" acuminata Ehrbg.
" labis Gosse.

Lepidoderma rhomboides Stokes
Dasydytes longisetosum Metschnikoff
" zelinkai Lauterborn.
" saltitans Stokes

Bryozoa

Plumatella repens (L.).
" (Alcyonella) fungosa (Pall.).

Mollusca

Limnaea (Gulnaria) auricularia L., characterized by resistance to some chemical sewage.
" auricularia f. ampla Hartm.
" ovata Drap.
Valvata piscinalis Müll.
Vivipara contecta Millet V. vera v. Frauenfeld, occurs abundantly also in foul-smelling mud.
" fasciata Müll.
Bythinia tentaculata (L.) Gray, also downstream from sewer outlets.
Lithoglyphus naticoides Ferussac in the Rhine River frequently associated with L. auricularia.
Neritina fluviatilis (L.), the egg capsules were frequently found on the shells of live paludines downstream from sewer outlets.
Unio tumidus Phil.
Sphaerium (=Cyclas) rivicolum Leach
" moenanum Kobelt
tends to be also oligosaprobiotic
Calyculina lacustris Müll.

Crustacea

Asellus aquaticus (L.) Ol., also alpha-mesosaprobiotic.
Gammarus fluviatilis Rös., also downstream from sewer outlets and also feeds on Spherotilus.
Cyclops strenuus S. Fischer, also alpha-mesosaprobiotic
Cyclops leuckarti Claus.
Cyclops brevicornis Claus, also alpha-mesosaprobiotic
Cyclops fimbriatus Fischer
Cyclops phaleratus Koch.
with their development stages
Diaptomus castor Jurine.
Canthocamptus staphylinus (Jur.), also in drinking-water sand-filters.
Cypridopsis vidua (O.F. Müll.).
Cypria ophthalmica Jurine.
Candona candida (Müll.) and other species
Daphnia pulex Degeer, also alpha-mesosaprobiotic.
" magna Strauss, "
" schaefferi Baird, "
" longispina O.F. Müll.
Moina rectirostris (F. Leydig).
Chydorus sphaericus (O.F. Müll.).
Pleuroxus excisus Schödler.

Hydrachnidae

Limnesia maculata (Müller) Bruzelius.
Arrhenurus bicuspidator Berl., also with Peranema and Euglena viridis.

Tardigrada

Macrobiotus macronyx Duj.

Neuroptera

Anabolia laevis Zett., larvae.
Molanna angustata Curtis, larvae.
Hydorpsyche angustipennis Curtis and larvae of some not accurately determined varieties.
Oxyethira costalis Curtis, larvae.

Diptera

Culex annulatus Fabr., and other species; larvae non-demanding.
Chironomus-larvae of a light yellow but not red color.
Ceratopogon-larvae of not accurately determined varieties.
Simulium ornatum Meig.
" reptans L.
also alpha-mesosaprobiotic.

Pisces (the most resistant representatives appear first)

Cobitis fossilis (L.).
Carassius carassius (L.).

Tinca tinca (L.).
Cyprinus carpio L.
Anguilla vulgaris Flem., with the exception of the youth stages.
Rhodeus amarus Bl.
Gasterosteus aculeatus L.
Leucaspius delineatus v. Sieb.
Alburnus lucidus Heck.

Amphibia

Rana esculenta L.
" fusca Rösel
spawn and tadpoles in part not very sensitive

III. Oligosaprobia

Rhizopoda

Amoeba proteus Leidy = A. princeps Ehrbg.
Difflugia globulosa Duj.,
" pyriformis Perty,
also in the beta-mesosaprobiotic zone.
" urceolata Cart.
" acuminata Ehrbg.
" corona Wallich, also in the beta-mesosaprobiotic zone.
" hydrostatica Zach.
" limnetica Levander and other species.
Lecquereusia spiralis (Ehrbg.)
Euglypha globosa (Cart.) Sphenoderia lenta Schlumbg.
Cyphoderia ampulla (Ehrbg.) Leidy.
Cyphidium aureolum Ehrbg.
Microgromia socialis Hertw. & Less, also in the beta-mesosaprobiotic zone.

Heliozoa

Rhaphidiophrys pallida F.E. Sch., also in the beta-mesosaprobiotic zone.
Acanthocystis turfacea Cart., also in the beta-mesosaprobiotic zone.

Flagellata

Dimorpha alternans Klebs.
Bicoeca lacustris J.-Cl.
" oculata Zach.
Diplosiga frequentissima Zach.
also in the beta-mesosaprobiotic zone.

Ciliata

Holophrya ovum Ehrbg., also in the beta-mesosaprobiotic zone.
Rhabdostyla ovum Kent.
Lacrymaria olor Ehrbg.
Trachelius elephantinus Svec.
Dileptus trachelioides Zach.
Ophryoglena atra Lieberk.
Frontonia acuminata (Ehrbg.) O. acuminata & atra Ehrbg.
Strombidium adhaerens Schew.
= Str. sulcatum Cl. & L.
" turbo Cl. & L., also in the beta-mesosaprobiotic zone.
Codonella lacustris Ents., also in the beta-mesosaprobiotic zone.
Oxytricha ferruginea Stein.
Stylonychia pustulata Ehrbg., also in the beta-mesosaprobiotic zone.
" histrio (O.F.Müll.).
Vorticella nebulifera Ehrbg.
Carchesium polypinum Ehrbg., also in the beta-mesosaprobiotic zone.
Ophrydium versatile Ehrbg.

Suctoria

Most representatives of this group are probably mesosaprobiotic except for Staurophrya elegans Zach.

Hydroidea

Cf. beta-mesosaprobiotic zone.
Cordylophora lacustris Allm.; lives mainly in brackish water.
Hydra vulgaris Pall. = H. grisea L.
" oligactis Pall. = fusca L.
" polypus L.
" viridis L.

Vermes

Haplotaxis gordioides (G.L. Hartm.)
= Phreoryctes menkeanus Hoffm.
Chaetogaster diaphanus (Gruith.), also in the beta-mesosaprobiotic zone.
Gordius aquaticus Duj.
Polycelis cornuta O. Schm.
Planaria gonocephala Dug.
Vortex pictus O. Schm., also in the beta-mesosaprobiotic zone.

Rotatoria and Gastrotricha

Floscularia cornuta Dobie.
Tubicolaria najas Ehrbg.
Asplanchna brightwelli Gosse
Sacculus viridis Gosse, also in the beta-mesosaprobiotic zone.
Triarthra breviseta Gosse
Rattulus capucinus Wierz. et Zach. = Mastigocerca hudsoni Lauterb., also in the beta-mesosaprobiotic zone.
Diurella stylata Eyf.
= Rattulus bicornis Western.
Salpina brevispina Ehrbg.
Euchlanis dilatata Ehrbg., also in the beta-mesosaprobiotic zone.
Pompholyx complanata Gosse.
Anuraea hypelasma Gosse.
Notholca foliacea Ehrbg., also in the beta-mesosaprobiotic zone.
" longispina Kellicott
" scapha Gosse.
Gastroschiza flexilis Jaegersk, also in the beta-mesosaprobiotic zone.
Ploesoma truncatum Levander, also in the beta-mesosaprobiotic zone.
Gastropus stylifer Imhof, also in the beta-mesosaprobiotic zone.
= Hudsonella pygmaca (Calm.).
Anapus ovalis Bergendal
" testudo Lauterb.
also in the beta-mesosaprobiotic zone.
Schizocerca diversicornis Dod., also in the beta-mesosaprobiotic zone.
Pedalion mirum Hudson
Ichthydium podura O.F. Muller
Chaetonotus maximus Ehrbg., occasionally also beta-mesosaprobiotic; frequent in dry wells; seems little sensitive to H_2S.
Chaetonotus larus O.F. Müller, occasionally also beta-mesosaprobiotic.

Bryozoa

Cristatella mucedo Cuv.
Fredericella sultana (Blumenb.) Gerv.
Paludicella ehrenbergi van Ben.

Mollusca

Limnaea stagnalis (L.) Lam.
" palustris Müll.
" peregra Müll.
also in the beta-mesosaprobiotic zone.
Amphipeplea glutinosa Müll.
Physa fontinalis (L.) Drap.
" acuta Drap.
Aplexa hypnorum L.
Planorbis corneus (L.)Pfeiff
also in the beta-mesosaprobiotic zone.

" marginatus Drap.
" carinatus Müll. and other kinds.
Ancylus fluviatilis Müll.
" lacustris L.
also in the beta-mesosaprobiotic zone.
Anodonta mutabilis Cless. Some varieties are very resistant.
Margaritana margaritifera L.
Unio pictorum L., often resistant.
" batavus Lam.
Pisidium amnicum Müll.
" fossarinum Cless.

Dreissensia polymorpha Pallas, especially typical
for this zone. larvae planktonic.

Crustacea

Astacus fluxiatilis Fabr.
Gammarus pulex (L.)De Geer.
Niphargus puteanus C.L. Koch.
Cyclops viridis Jur.
" albidus Jur.
" serrulatus Fischer, also in the beta-
mesosaprobiotic zone.
" bicuspidatus Claus.
" fuscus Jur.
" oithonoides Sars.
Diaptomus gracilis Sars.
" graciloides Lilljeborg.
" laciniatus Lillj.
Eurytemora velox (Lillj.).
Canthocamptus minutus Claus. also
Cypris virens Jurine). in the beta-meso-
" incongruens (Ramdohr) saprobiotic zone.
Sida cristallina (O.F. Mull.).
Diaphanosoma brachyurum (Liévin).
" leuchtenbergianum S. Fischer.
Holopedium gibberum Zaddach.
Daphnia hyalina Leydig with subspecies galeata Sars
" (Hyalodaphnia) cucullata G. O. Sars
kahlbergiensis Schoedler.
Scalopholeberis mucronata (O.F. Mull.).
Simocephalus vetulus (O.F. Mull.) Schoedler.
Ceriodaphnia reticulata (Jur.), also in the beta-
mesosaprobiotic zone.
Bosmina longirostris (O.F. Mull.); P.E. Mull. & var.
cornuta Jur., also in the beta-meso-
saprobiotic zone.
" coregoni Baird.
" " var. gibbera Schoedler.
Acroperus harpae Baird.
Leidigia quadrangularis (Leydig) also in the beta-
mesosaprobiotic zone.
Lynceus (Alona) guttatus (Sars.); also in the beta-
mesosaprobiotic zone.
" costatus (Sars.); and other species.

Bythotrephes longimanus Leydig.
Leptodora kindti (Focke).

Argyronetidae

Argyroneta aquatica Cl.

Hydrachnidae

Most representatives belong in this zone.
Atax crassipes O.F. Mull. (Bruzelius).
Neumania spinipes Mull.
Curvipes nodatus Mull.
" rufus C.L. Koch.
Hygrobates nigro-maculatus Lebert.
Limnochares holosericea Latreille.

Orthoptera. Larvae.

Libellula depressa L., and other species.
Aeschna grandis L.
Calopteryx virgo L., also in the beta-meso-
saprobiotic zone.
Agrion puella L.
Ephemera vulgata L.
Polymytarcis (Palingenia) virgo Ol.
Prosopistoma foliaceum Fourcroy
Baetis species
Heptagenia (Ecdyurus) fluminum Pict.
Cloe diptera L., also in the beta-meso-
saprobiotic zone.
Perla bicaudata L.
" nubecula Newm.
Taeniopteryx trifasciata Pict.
Nemura variegata Oliv.

Neuroptera

Phryganea striata L., larvae.
" grandis L., "
Sericostoma, larvae of different species;
also beta-mesosaprobiotic.
Brachycentrus subnubilus Curt.; also in the beta-
mesosaprobiotic zone.
Leptocerus annulicornis Steph.
Rhyacophila vulgaris Pict.
Hydroptila sparsa Curt.

Hemiptera

Hydrometra lacustris L. not very suitable
" rufoscutellata Cuv. for
Limnobates stagnorum Cuv. water evaluation
Nepa cinera L., rather sensitive to lack of oxygen
Ranatra linearis L., also in the beta-
mesosaprobiotic zone.
Aphelocheirus aestivalis Fabr.
Corixa striata L., appears with lack of oxygen under
ice first at the "Wuhnen" (colloquial term,
possibly meaning hole?).
Notonecta glauca L., somewhat less sensitive to
lack of oxygen than Corixa.

Diptera

Corethra plumicornis Fabr., larvae very resistant.

Coleoptera

Dytiscus marginalis L., larvae and beetles; able to
follow prey into mesosaprobiotic zone like
other predators.
Acilius sulcatus L., larvae and beetles.
Colymbetes fuscus L., larvae and beetles.
Agabus bipustulatus L., larvae and beetles.
Gyrinus natator L., larvae and beetles, not very
suitable for water evaluation.
Hydrophilus piceus L., larvae and beetles.

Pisces (the most sensitive representatives are listed
first).

Gasterosteus pungitius L.
Esox lucius L.
Lota vulgaris Cuv.
Gobio fluviatilis Cuv.
Scardinius erythropthalmus L.
Blicca björkna L.

Lucioperca sandra L.

Acerina cernua L.
Idus melanotus Heck. & Kn.
Abramis brama L.

Leuciscus rutilus L.
Perca fluviatilis L.
Trutta fario L.

Amphibia

Triton cristatus Laur.
" *taeniatus* Schneid.

Literature References

Note: The Institute referred to in the text is Kgl. Prufungsanstalt fur Wasserversorgung und Abwasserbeseitigung (Royal Institute for Water Supply and Sewage Disposal).

Note: A number of reports (here given in chronological order) listed below are not specifically referred to in the text but are intended merely as a collection of the widely dispersed pertinent literature.

(1) Lindau, Schiemenz, Marsson, Elsner, Proskauer, Thiesing: Hydrobiological and Hydrochemical Investigations on the Collector Systems of the Bake, Nuthe, Panke and Schwarze. Vol XXI, 1901, Supplement.

(2) Marsson and Schiemenz: Damage to Fishing in the Penne River Through the Sugarmill in Anklam. Vol IX, 1901. No. 1.

(3) Lauterborn, R: Contributions to the Microfauna and Flora of the Moselle River with Special Consideration of Sewage Organisms. Vol. IX, 1901.

(4) Lauterborn, R: The Sapropelic Fauna. Vol. XXIV, 1901.

(5) Kolkwitz and Marsson: Principles for Water Evaluation from Its Fauna and Flora. 1902, No. 1.

(6) Marsson, M: Fauna and Flora of Contaminated Water and Their Inter-relation to Biological Analysis of Water. Vol. X, 1903.

(7) Volk, Rich: Investigation of the Elbe River at Hamburg. Vols XVIII, XIX, XXIII, 1901-1906.

(8) Schiemenz: Organic Sewage Examined in Relation to Fishing. 1901-1907.

(9) Marsson, M: Flora and Fauna of Some Sewage Treatment Stations at Berlin and Their Significance for the Purification of Municipal Sewage. 1904, No. 4.

(10) Marsson, Spitta, Thumm: Expertise on the Permissibility of the Discharge of Fecal Matter into the Main River by the City of Hanau. 1904, No. 5.

(11) Kolkwitz and Thiesing: Chemobiological Investigations on the Use of Trickle Fields for the Purification of Stow-Dam Water for Human Consumption. 1904, No. 5

(12) Schiemenz: Evaluation of the Purity of Surface Water from Macroscopic Animals and Plants. Vol. XLIX, 1906.

(13) Selk, H: The Algae of the Elbe River and Its Estuary. Vol. XXV, 1907

(14) Volk, Rich: The Biological Investigation of the Elbe River by the Hamburg Natural History Museum. Vol. XV, 1907.

(15) Kolkwitz, R: Biological Self-Purification and Evaluation of Waters. No. 2, 1907.

(16) Kolkwitz, R: Biology of Caverns, Sources and Wells. No 37, 1907.

(17) Kolkwitz, R: Instruments for Sampling and Observation in Biological Examinations of Water. No. 9, 1907.

(18) Kolkwitz and Ehrlich: Chemobiological Investigations of the Elbe and Saale Rivers. No. 9, 1907.

(19) Lemmermann: Flagellatae. Vol. III, 1907.

(20) Marsson, M: Four Reports on the Biological Investigation of the Rhine River Between Mayence and Coblentz. 1907 and 1908.

(21) Lauterborn, R: Four Reports on the Biological Investigation of the Rhine River Between Basel and Mayence. 1907 and 1908.

(22) Kolkwitz and Marsson: Ecology of Plant Saprobia. Vol. XXVIa, 1908, pp. 505-519.

(23) Kolkwitz, Pritzkow and Schiemenz: Two Expertises on the Sewage and Outflow Conditions of a Cellulose Plant Near Kattowitz. No. 10, 1908.

Reproduced With Permission From:
THE PROGRESSIVE FISH-CULTURIST
11(1949): 217-230

VALUE OF THE BOTTOM SAMPLER IN DEMONSTRATING THE EFFECTS OF POLLUTION ON FISH-FOOD ORGANISMS AND FISH IN THE SHENANDOAH RIVER

Crosswell Henderson
U.S. Fish and Wildlife Service
Kearneysville, West Virginia

IN THE PAST several years legislation and activity directed toward the control and abatement of stream polution have greatly increased. Numerous States have passed legislation forming water commissions, water-control boards, or other groups or committees to deal with stream pollution within their borders. Some States have formed interstate agencies to deal with activities in a particular river basin. The passage of Public Law 845 greatly increased activities from a national viewpoint.

All these groups have at sometime or another been concerned with stream surveys to determine extent or degree of pollution. Many groups have worked on this problem and have come up with varied answers. Some have set arbitrary standards of cleanliness, below which no body of water would be allowed to degrade. Others have classified streams into groups (A, B, C, D, etc.) depending on usage and have set standards of cleanliness to be maintained in each class. Still others have set no standards but have treated each pollution problem separately and have had a board to decide the degree of pollution that would be allowed. Unfortunately, little or no consideration has been given to fish or wildlife in many of these surveys or classifications. For many years sportsmen's groups have been fighting for clean streams, and these groups have been instrumental in causing the enactment of much of the constructive pollution legislation. Yet, when pollution is discussed at various meetings and standards are set for streams, practically nothing is heard about what aquatic life (other than bacteria) is in, or should be maintained in a stream.

For a great many streams there is a wealth of information as to the coliform bacteria count, the biochemical oxygen demand, total solids, color, odor, and other factors; but there is very little information as to what fish or fish-food organisms may be present. True, most of these pollution surveys have been made from the standpoint of public health or for municipal or industrial water supplies--which are, of course, very important. But why not include in these surveys some data as to the effects of pollution on normal aquatic life?

Requirements for water to drink or to operate an industry may be vastly different from the requirements for the maintenance of fish and aquatic life. Water from an open sewer would certainly be unfit to drink but, if not in such excessive quantity as to use all the oxygen in the receiving stream, may even have a beneficial effect (through fertilizer values) on aquatic life. Water suitable for industrial use may have small quantities of some toxic substance which would make that water deadly to aquatic life.

Aquatic life in streams may be depleted generally in four ways: (1) lack of dissolved oxygen, (2) too high or too low hydrogen-ion concentration, (3) smothering effect of silt or other fine material, (4) the presence of definitely toxic substances. In general, most pollution surveys would demonstrate the first two of these conditions. Dissolved oxygen and pH are standard tests used in pollution surveys, and the requirements of most aquatic animals are known. The third may show up in observations or from turbidity determinations. The fourth condition would become apparent only through elaborate chemical tests and a knowledge of the toxicity of numerous substances to the various aquatic animals.

Simple methods of determining the effects of the third and fourth factors are based upon use of a Surber (1937) square-foot bottom sampler (figure 1) in gravel and rubble, and Ekman or Peterson dredges in mud or silt. Stream bottoms are normally the habitat for numerous aquatic insect larvae and other aquatic animals. These forms may include the larvae of May flies (Ephemerida), stone flies (Plecoptera), dragonflies (Anisoptera), damsel flies (Zygoptera), midge flies and crane flies (Diptera), caddis flies (Trichoptera), dobson flies (Neuroptera), and other aquatic animals such as water beetles (Coleoptera), aquatic earthworms (Oligochaeta), crayfish and shrimp (Crustacea), and snails and clams (Mollusca). These are the principal fish-food organisms. Square foot bottom samples taken in riffles above and below sources of pollution show immediately the approximate quantity and the types of fish-food organisms present. Without sufficient quantities of these animals, fish soon disappear for lack of food, whether they can survive in the water or not.

Though all of the physical, chemical, and bacteriological determinations normally used in any stream evaluation are quite important, there is frequently a definite lack of biological determination to show the actual impact of pollution on aquatic life in the stream. This has been forcibly pointed out by Beatty (1947). Ellis and Westfall (1946) and Hart, Doudoroff, and Greenbank (1945) established uniform bioassays or toxicity experiments which could be used to show toxicity levels of various chemicals and wastes for fishes. Though such tests are very useful, there may be great differences between laboratory toxicity tests and actual conditions in streams. Other workers, including Platner (1946), Wiebe (1928), and Ellis (1940), used Peterson dredges and bottom samplers of various kinds to determine the biological impact of pollution.

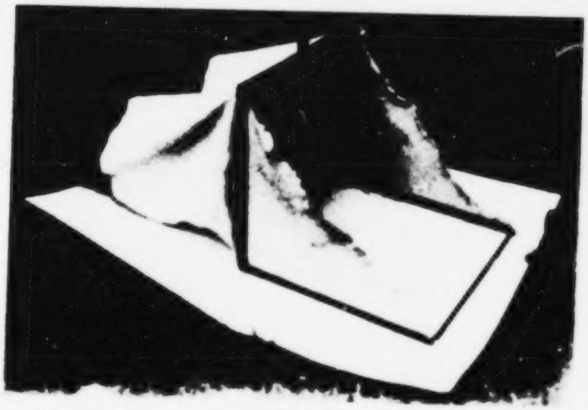


Figure 1 - Surber square-foot bottom sampler.

Methods for the collection and microscopical examination of plankton have been known and used for many years. Many workers, including Lackey (1940), McGauhey (1942), and Purdy (1930), have applied these methods to pollution studies, in which certain types of protozoan plankton and other microscopic organisms were used as indicators of stream pollution.

In a study of pollution of the Coeur D'Alene River, Ellis (1940) called attention to the presence of large numbers of bottom animals, such as caddis-fly larvae and stone-fly and May-fly nymphs, in riffle areas above sources of pollution -- and their complete absence below these sources. Surber (1939) used a square-foot bottom sampler of a new, compact design in sampling four eastern smallmouth-bass streams to determine the fish-food organisms present. This work led to the use of this bottom sampler in pollution studies in the Shenandoah River.

PURPOSE OF INVESTIGATIONS

The Shenandoah River was for a long time the favorite fishing ground of numerous residents of northern Virginia and the eastern panhandle of West Virginia. In fact, this river was so noted as a smallmouth-bass stream that sportsmen were attracted from all over the eastern United States to try their luck at catching the elusive bass. In 1940, a large corporation, the American Viscose Company, began operations at Front Royal, Virginia, on the South Fork of the Shenandoah just above the confluence with the North Fork. Though a clean stream was promised the sportsmen of this section, it was only a short time until fishing in the main Shenandoah had declined considerably. Numerous dead fish were seen in the river during the winter of 1942-43. Sportsmen became alarmed and called upon the Fish and Wildlife Service to determine the condition of the river. Bottom samples taken above and below the Viscose plant showed more than 99 percent decrease in bottom animals 30 miles below the plant, as compared with the number immediately above. This fact was reported to the Virginia Commission of Game and Inland Fisheries in August 1943, but little could be done because of very weak pollution laws. Further fish kills were observed in the winters of 1943-44 and 1944-45, when sport fishing had practically ceased to exist on the main river. About this time, Izaak Walton League chapters were formed in Winchester and Berryville, Virginia, with the clean-up of the Shenandoah River their main objective. These and other nearby chapters sponsored an investigation to determine sources and degree of pollution and its effects on aquatic life in the river: the facts obtained in this study were to be used in making the general public aware of existing conditions. This study was started in June 1947 and was continued throughout the summer. This survey was continued through 1948 by the Fish and Wildlife Service from its Fishery Station at Leetown, West Virginia. Investigations are still under way. This paper is not a complete report of work on the Shenandoah but is undertaken at this time to show certain correlations of numbers and types of bottom animals to pollution, and to show the desirability of using the Surber bottom sampler more widely as a tool in stream pollution surveys.

DESCRIPTION OF RIVER

The Shenandoah River (figure 2) rises in the mountains of northern Virginia, the North Fork in the Allegheny Mountains and the South Fork in the Blue Ridge. Massanutten Mountain separates the two forks. Each fork, fed intermittently by mountain streams and limestone springs, flows a distance of some 150 miles through fertile limestone valleys to Front Royal, Virginia. From the confluence of the forks at Front Royal, the main Shenandoah flows north about 60 miles along the foot of the Blue Ridge Mountains, entering the Potomac River at Harpers Ferry, West Virginia. The lower 20 miles of the river are in West Virginia.

This river is normally a clear, fast-flowing, fertile stream. Extensive riffle areas and limestone ledges serve to make ideal food and cover conditions for fish.

Surber, comparing the Shenandoah River (main stream) with other smallmouth-bass streams in this area (1939), found the growth of bass to be very rapid: the bass reached legal length (10 inches) in 2 to 3 years. Food conditions were reported to be excellent, as there was an abundance of forage fish and bottom animals.

Prior to 1940, the whole Shanandoah River system was considered a mecca for fishermen. Residents of

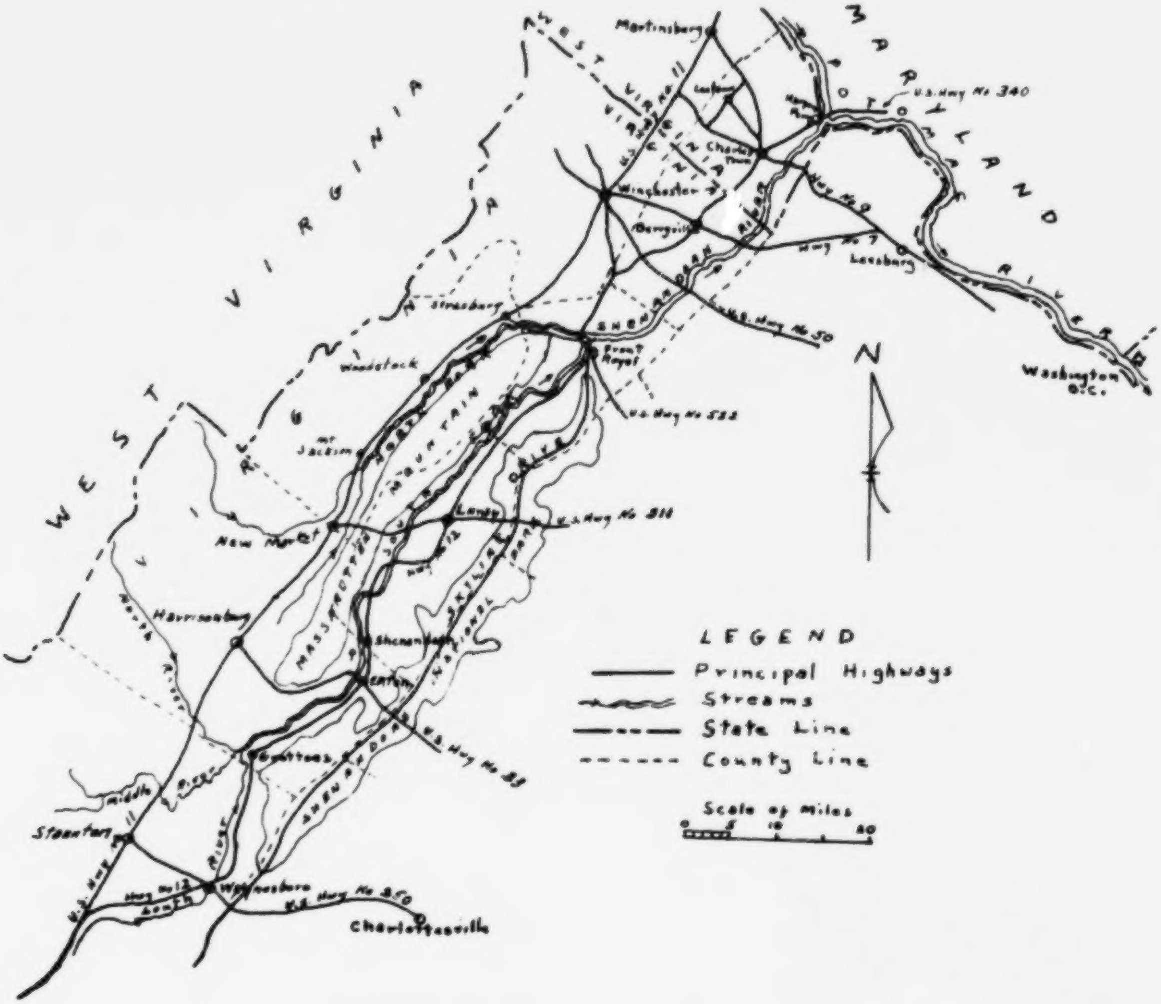


Figure 2 - The Shenandoah River System.

this area claim that as a smallmouth-bass stream it could not be surpassed. Excellent fishing occurred in practically all of the river. The principal species taken were smallmouth bass, largemouth bass, yellowbelly sunfish, channel catfish, carp, suckers, and crappie.

POLLUTION IN THE SHENANDOAH RIVER SYSTEM

Preliminary surveys disclosed no serious sources of pollution (from the fishery standpoint) in the North Fork. Several small towns (Woodstock, Strasburg, and others) discharged raw sewage into the river, but the dilution factor was such that there were no detrimental effects to aquatic life; so the North Fork was not included in this survey.

Three streams--South River, Middle River, and North River--meet just above Port Republic, Virginia, to form the South Fork of the Shenandoah. At Waynesboro, South River receives a serious load of pollution from industries and sewage from the town itself. Fish kills have been reported to the Virginia Game Commission several times. At present, the Virginia Water Control Board is making a study of this part of the river. Owing to this and to the fact that this portion of the river was not considered important for sport fishing, it was not included in this investigation. The remainder of the Shenandoah River system was included (figure 3).

Preliminary bottom samples showed no apparent effect on aquatic life from the sewage of the four towns; so this study was confined primarily to the effect of industrial wastes from the Merck Chemical Company and the American Viscose Corporation plants.

It was suspected that the Viscose effluent contained one or more substances that were deadly to aquatic animals and that this material persisted in the water for a considerable distance downstream. From a study of materials used at the Viscose Corporation plant in

Figure 3 - Study stations and sources of pollution.

Sources of pollution:

A -- The Merck Chemical Company, Elkton, Virginia: manufactures streptomycin, vitamin B_1, and other chemicals; empties wastes (nature of which is not definitely known) directly into the South Fork of the Shenandoah River; has facilities for waste treatment.

B -- Domestic sewage from the town of Elkton, Virginia: population, 1,500; untreated wastes empty directly into the river.

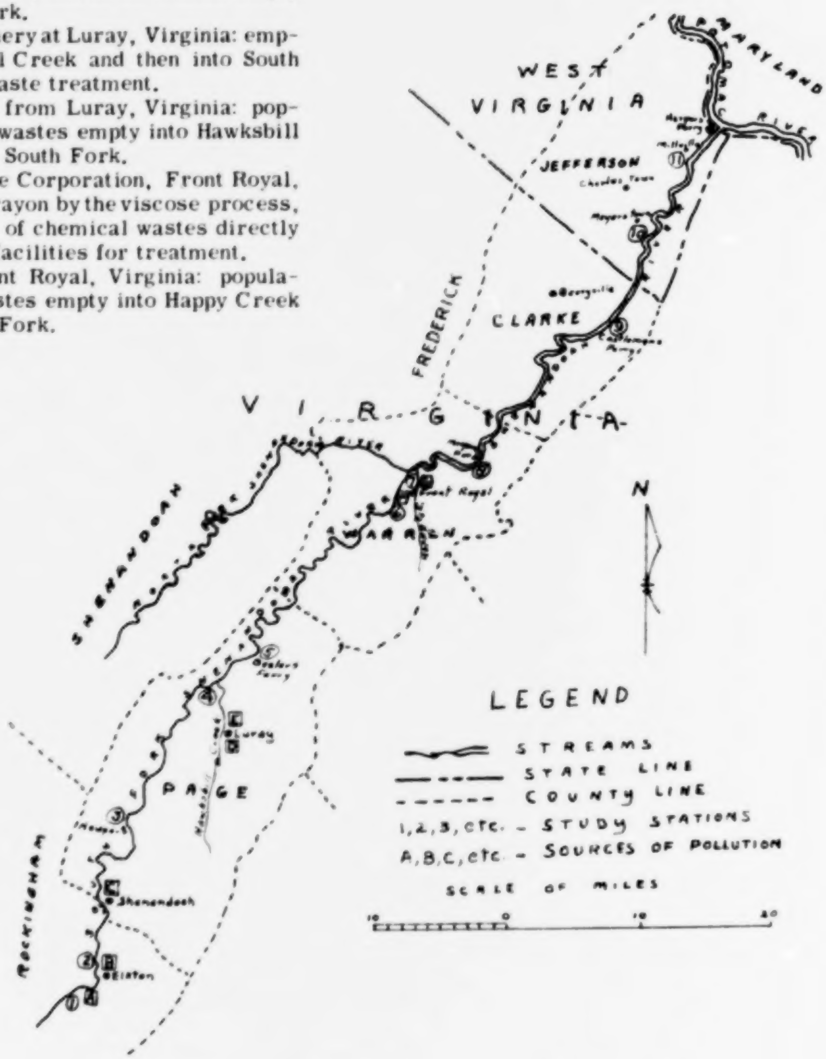
C -- Domestic sewage from the town of Shenandoah, Virginia: population, 1,000; untreated wastes empty directly into the South Fork.

D -- Virginia Oak Tannery at Luray, Virginia: empties wastes into Hawksbill Creek and then into South Fork; has facilities for waste treatment.

E -- Domestic sewage from Luray, Virginia: population, 1,100; untreated wastes empty into Hawksbill Creek and thence into the South Fork.

F -- American Viscose Corporation, Front Royal, Virginia: manufactures rayon by the viscose process, empties large quantities of chemical wastes directly into the South Fork, has facilities for treatment.

G -- Sewage from Front Royal, Virginia: population, 5,200; untreated wastes empty into Happy Creek and thence into the South Fork.



making rayon by the Viscose process (Roetman 1944) and a review of the literature concerning toxicity of various substances to aquatic animals (Ellis 1937), several substances -- among them, xanthates, hydrogen sulphide, and zinc -- were immediately suspected. Xanthates were largely eliminated because of assurance by Viscose Corporation officials that this material could not possibly reach the river and because xanthates could not exist in the effluent under acid conditions. (The effluent was highly acid (pH 2.4) at least at times during the period of major destruction of aquatic animals in the river.) Hydrogen sulphide, though present in toxic quantities in the effluent, was probably eliminated as a result of aeration in the riffle areas of this fast-flowing river. Analysis of the viscose effluent showed zinc to be present in quantities above 2,000 parts per million. In England several workers with lead and zinc mine pollution had shown zinc in quantities as low as 0.2 parts per million to be lethal to some aquatic animals (Newton 1944). Analysis of the water below the Viscose Corporation showed zinc to be present in quantities considerably above this value: tests revealed the presence of zinc at the rate of 0.4 to 11 parts per million, depending on river flow and other conditions. Zinc was thus indicated as at least one of the major factors contributing to the destruction of aquatic life in the river.

Laboratory toxicity experiments were made with both Viscose effluent and pure zinc sulphate to determine the effects on fish and other aquatic animals. It was determined that the Viscose effluent (in concentrations simulating low water and normal river conditions) was deadly to at least some kinds of aquatic animals, and that zinc itself (in quantities present in the river) was toxic to some aquatic animals, such as bass fry, daphnia, and snails. The results of this work with zinc led Viscose officials to set up their own toxicity work with zinc and to put major emphasis on the elimination of zinc from their effluent.

Since the construction of the Viscose Corporation plant in 1940, that organization has expended considerable amounts of effort and money to work out processes for treatment of waste (Roetman 1944). Until 1948, however, the Viscose effluent was treated only a part of the time, and this treatment was entirely inadequate insofar as the aquatic life in the river was concerned. As a result of the publicity received in connection with this study that showed the river to be virtually devoid of aquatic life, the Viscose Corporation has made further effort to improve the treatment of its effluent and has done additional work in reducing the zinc content of that effluent. Reports (April 6, and December 6, 1948) to the Virginia Water Control Board showed much improvement in treating the effluent. At the present writing (June 1949) considerable progress seems to have been made. Complete treatment (ordered by the Virginia Water Control Board to be in effect by March 1949) has been in effect for some time, and the river appears to be recovering.

SAMPLING STATIONS

Eleven study stations (table 1, figure 3) were established at accessible riffle areas above and below possible sources of pollution. Physical, chemical, and biological determinations were made at each station once each month from June to October 1947, and in June and September 1948. These determinations were made at low-water or normal river stages.

PHYSICAL AND CHEMICAL CONDITIONS

Water temperature, hydrogen-ion concentration, dissolved oxygen, free carbon dioxide, and methyl-orange alkalinity (table 2) were determined by standard methods of water analysis (American Public Health Association 1946). Other physical and chemical data were obtained from the West Virginia Water Commission (table 3).

BIOLOGICAL CONDITIONS

In riffle areas near each station, bottom samples were taken with a square-foot bottom sampler used in the manner described by Davis (1938). Square-foot bottom samples were collected, sieved, and preserved in formalin. The animals were later picked out, identified to order, counted, and weighed (wet weight after draining 45 seconds). (See tables 4 and 5.)

ANALYSIS OF DATA

Physical and chemical conditions in the Shenandoah River (tables 2 and 3) are such that by accepted standards (Ellis 1937) the water would be deemed satisfactory to support nearly all forms of aquatic life. In only a very few instances, and then only for short distances below sources of pollution, were dissolved oxygen and pH values such that a good mixed aquatic fauna could not be maintained. Dissolved oxygen values as low as 3.9 parts per million occurred at Station 2 (3 miles below Merck Company) during low water in the summer of 1947 and averaged 5.9 parts per million for the summer, but at Station 3 (less than 20 miles downstream) dissolved oxygen values averaged 8.4 parts per million and at no time were less than 6 parts per million. At no time was a value for dissolved oxygen at Station 1 (above Merck Company) found below 6 parts per million. This tended to show a definite oxygen demand by Merck Company wastes and indicated that conditions below would be somewhat hazardous to certain organisms requiring high oxygen content of water. Bottom-animal samples (table 4, figure 4) appeared to substantiate this belief. Samples at Station 1 contained an average of 912 bottom animals weighing 6.5 grams, while samples at Station 2 averaged only 108 bottom animals weighing 0.42 gram. Samples at Station 3 (25 miles below Merck Company) averaged 528 bottom animals weighing 4.13 grams. A major portion of the bottom animals at Station 2 consisted of oligochaete worms, leeches, and midgefly larvae (animals having low oxygen requirements); while at Stations 1 and 3 there was an abundance of caddis-fly larvae and May-fly nymphs and other types (table 5) which generally require high oxygen content of the water.

Reports from fishermen also tend to substantiate this conclusion. Though carp (fish having low oxygen

TABLE 1.--Station numbers, approximate location, miles between stations, and stream-flow data

Station No.	Location of stations	Miles from Station 1	Stream flow[1/] cubic feet per second 1930-42 average Ave.	Min.	Min. daily
	South Fork, Shenandoah				
1	1 mile above Merck Co., 4 miles south of Elkton, Va.	-	974	32	93
2	3 miles below Merck Co., Elkton, Va.	4	-	-	-
3	25 miles below Merck Co., Newport, Va.	26	-	-	-
4	1 mile above Hawksbill Creek, Shenandoah Lodge, Va.	43	-	70	135
5	5 miles below Hawksbill Creek, Beelers Ferry, Va.	50	-	-	-
6	1 mile above Viscose Co., Front Royal, Va.	86	-	-	-
7	1 mile below Viscose Co., Riverton, Va.	88	1,713	59	103
	Shenandoah River				
8	10 miles below Viscose Co., Morgans Ford	97	-	-	-
9	30 miles below Viscose Co., Castlemans Ferry	119	-	-	-
10	40 miles below Viscose Co., Meyerstown, W. Va.	129	-	-	-
11	50 miles below Viscose Co., Millville, W. Va.	137	2,453	59	194

[1/] Stream-flow data from Geological Survey Water Supply Paper 951.

requirement) were caught quite often in the vicinity of Station 2, few bass were taken. On the other hand, reports showed bass fishing fair to good near Stations 1 and 3.

The low oxygen conditions existing in the summer of 1947 have evidently been corrected. Samples taken in 1948 (table 2) show dissolved oxygen values well above those expected to support a good mixed aquatic fauna. Bottom animals (table 4) have also shown a remarkable recovery in this section of the river and are now present in numbers and weights similar to what would be expected in unpolluted sections of this stream. Certain of the important types such as May-fly nymphs, however, have not yet appeared (table 5). Caddis-fly larvae and hellgrammites have become very abundant.

From Station 3 to Station 6, the river shows little variation in physical and chemical qualities (table 2). All values are well within the limits that may be expected to support a good aquatic fauna. Bottom animals of types expected were abundant in all riffle areas examined in this section of the river (tables 4 and 5). At the beginning of this study, it was expected that wastes from the tannery and sewage from Luray would have some detrimental effect on the river. Field tests, however, did not substantiate this idea. Chemical and biological conditions appeared as good below this possible source of pollution as above. Consequently, Station 4 was eliminated as a study station. Fish kills in Hawksbill Creek were reported to the Virginia Game Commission late in 1947, but no detrimental effects were shown in the Shenandoah, possibly because of dilution.

TABLE 2.--Average, maximum, and minimum values of temperature, dissolved oxygen, pH, free carbon dioxide, and methyl-orange alkalinity, Shenandoah River

Station No.	Water temperature (°F.)			Dissolved oxygen (p.p.m.)			pH			Free carbon dioxide (p.p.m.)			Methyl-orange alkalinity (p.p.m.)
	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
	1938 (May 1 to October 31, 5 samples)[1/]												
9	71			9.0			8.2			0.5			116.5
	1947 (June to October, 5 samples)												
1	69	76	54	7.5	10.3	6.1	7.9	8.0	7.9	2	5	0	
2	71	77	56	5.9	8.7	3.9	7.7	7.9	7.5	3	2	6	
3	72	80	56	8.4	10.6	6.0	7.9	8.0	7.7	0	0	0	
5	74	81	64	9.8	11.3	9.1	8.3	8.6	8.0	0	0	0	
6	76	85	64	10.3	12.8	8.1	8.6	9.2	8.2	0	1	0	
7	79	89	66	7.2	9.4	4.4	5.8	7.4	4.4	65	94	10	
8	78	87	64	8.5	9.4	6.3	7.9	8.2	7.2	0	0	0	
9	78	88	62	8.6	9.6	6.0	8.0	7.7	8.2				
10	-	88	-	8.2	-	-	7.9	-	-				
11	-	88	-	7.7	-	-	8.1	-	-				
	1948 (June 23 and September 29, 2 samples)												
		June	Sept.		Sept.	June		June	Sept.		June	Sept.	
1	68	74	61	8.2	9.1	7.4	8.0	8.1	8.0	0	0	0	145
2	68	74	62	7.0	7.9	6.0	8.0	8.0	7.9	1	2	1	140
3	70	78	62	9.1	9.2	9.0	8.4	8.8	8.0	0	0	0	130
5	71	80	63	9.4	10.0	8.8	8.3	8.5	8.2	0	0	0	131
6	75	82	68	10.2	11.0	9.4	8.5	8.7	8.3	0	0	0	121
7	77	85	69	9.2	10.2	8.2	8.0	8.3	7.7	5	10	0	108
8	75	82	68	9.3	9.9	8.8	8.3	8.7	8.0	0	0	0	126
9	72	79	66	7.9	9.1	6.8	8.2	8.2	8.2	0	0	0	114
10	74	81	64	8.6	9.2	8.1	8.3	8.5	8.1	0	0	0	125
11	76	87	66	8.1	9.0	7.3	8.3	8.5	8.0	0	0	0	123

[1/] Surber, E. W. (1938 data).

TABLE 3.--Shenandoah River stream-survey data furnished by West Virginia Water Commission

	Source of sample								
	Point 1[1]			Point 2[2]			Point 3[3]		
Date of sample (1947)	8/29	9/5	9/12	8/29	9/5	9/12	8/29	9/5	9/12
Laboratory number	44	44	44	43	43	43	42	42	42
Water temperature, °F.	80	79	82	82	78	80	80	80	85
Dissolved oxygen,									
p.p.m.	9.7	7.65	9.75	8.25	7.35	7.7	7.25	6.95	7.9
percent saturation	119	93	122	104	89	95	89	85.5	102
Bio-chemical oxygen demand, p.p.m.	0.5	0.7	0.85	0.5	0.75	1.2	0.5	0.6	1.5
Hydrogen-ion concentration (pH)	8.15	8	8.35	8.28	8.45	8.35	8.2	8.2	8.4
Alkalinity[4]									
methyl orange	100	112	116	117	119	115	121	120	111.5
phenolphthalein	-0.5	-0.5	2	Neut.	5	2.5	Neut.	3	1.5
Chlorides, p.p.m.	10.5	10	14	10	11	12	11.5	10.5	11
Hardness, p.p.m.	141	162	171	149	152	156	160	166	170
M.P. No. Coliform organisms X 1000	<0.11	0.026	<0.11	<1.1	0.026	0.11	<1.1	1.5	0.89

[1] Rt. 7, east of Berryville, Virginia. Corresponds to Station 9.

[2] Rt. 9, east of Charles Town, West Virginia. Corresponds to Station 10.

[3] Millville, West Virginia. Corresponds to Station 11.

[4] Negative alkalinity indicates acidity.

TABLE 4.--Average, maximum, and minimum numbers of bottom animals at each station

Station No.	Bottom animals						
	Number per square foot			Grams per square foot			Pounds per acre
	Ave.	Max.	Min.	Ave.	Max.	Min.	Ave.
	1936 (August, 10 samples)						
9	203			10.05			942
	1943 (August, 5 samples)						
6	417			7.45			698
9	4			0.08			7
	1947 (June to October, 5 samples)						
1	912	1,420	340	6.50	10.25	2.18	611
Merck Co.							
2	108	187	78	0.42	1.05	0.18	40
3	528	634	440	4.13	5.01	2.75	390
5	628	866	466	10.55	13.90	4.76	990
6	590	781	325	18.56	23.60	14.45	1,752
Viscose Co.							
7	0	0	0	0	0	0	0
8	70	221	1	0.62	1.50	0.10	59
9	32	92	5	0.12	0.20	0.01	11
10[1]	2			0.05			5
11[2]	19	20	18	0.10	0.10	0.10	10
	1948 (June and September, 2 samples)						
1	1,362	1,469J[3]	1,255S[4]	6.45	7.06J	5.83S	604
2	560	871S	250J	4.07	7.94S	0.20J	381
3	566	675J	458S	1.97	2.39J	1.55S	185
5	714	927S	501J	18.95	20.78J	17.13S	1,776
6	937	1,200S	674J	17.60	27.43S	7.78J	1,649
7	0	0	0	0	0	0	0
8	85	150J	20S	0.29	0.33S	0.25J	27
9	270	367J	172S	0.32	0.42S	0.23J	30
10	187	301J	74S	0.40	0.69J	0.10S	37
11	362	600J	124S	0.64	0.80J	0.48S	60

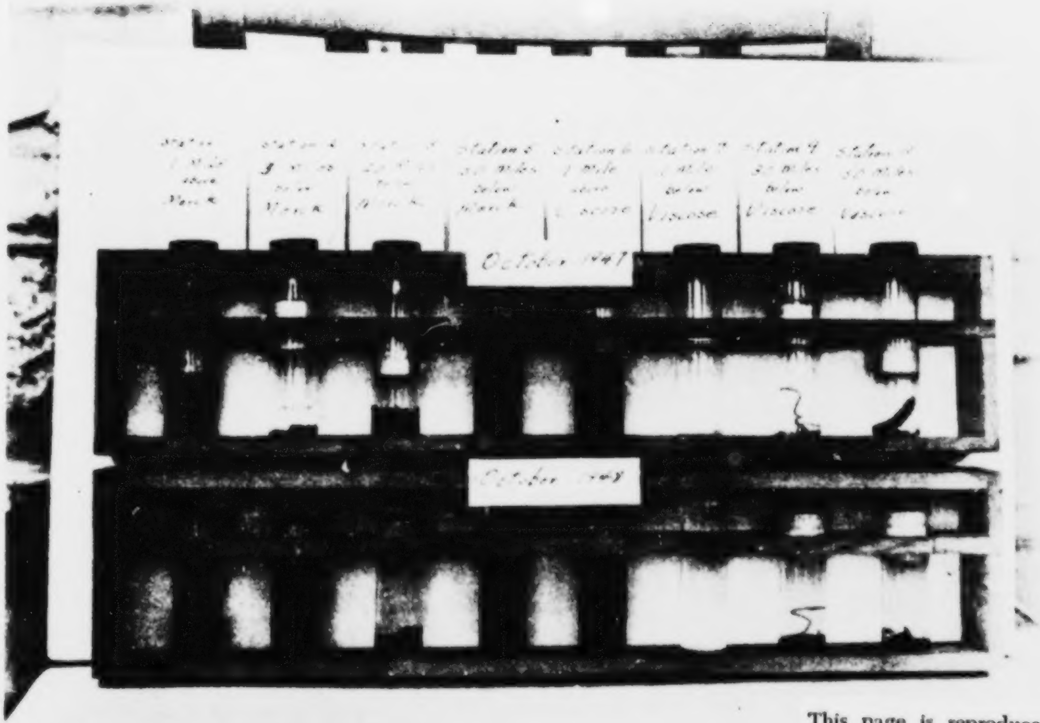
[1] One sample only.
[2] Two samples only.
[3] J - June.
[4] S - September.

This section from Station 3 to Station 6 (approximately 60 miles) is known far and wide as an excellent smallmouth bass stream. Many excellent catches of bass, as well as sunfish, channel catfish, and other fishes, are taken during the fishing season.

From Station 7 to its mouth (55 miles), the Shenandoah presents an entirely different picture. Although standard physical, chemical, and bacteriological tests (tables 2 and 3) showed nothing seriously wrong, bottom samples taken in the summer of 1947 (table 4) showed this section of the river to be virtually devoid of aquatic life.

It is possible that, while this section (Station 7 to the mouth) is wholly unfit for fishing, values obtained from standard stream surveys (table 3) would place it in a very high category (Class A, West Virginia Water Commission, 1948). Bottom samples (table 4), however, have shown the marked depletion of aquatic life in this stream. At Stations 8, 9, 10, and 11, values for temperature, dissolved oxygen, pH, turbidity, and carbon dioxide (table 2) have consistently remained within limits that may be expected to support a good aquatic fauna. At Station 7, values for dissolved oxygen and pH were below recognized limits for a short time in the summer of 1947 but have since remained within the desired limits.

Bottom samples (tables 4 and 5) have shown that, though bottom animals of most desirable types were abundant at Station 6 (1 mile above Viscose), in no instances were any bottom animals found at Station 7 (1 mile below Viscose). Samples taken at Stations 8, 9, 10, and 11 showed bottom animals to be extremely limited in both number and desirable type (figure 4).



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 4 - Bottom animals in square-foot samples.

In August 1943, examining 5 bottom samples taken at Station 6 and 10 samples taken at Station 9 (table 4), Surber found bottom animals at Station 9 reduced 99 percent in average number and 97 percent in weight. The data from Station 9, as compared with data taken at the same station in 1936 (before pollution), showed a reduction of 98 percent in average number and 99 percent in average weight of bottom animals.

Bottom samples taken in the summer of 1947 showed practically the same conditions existing then as in 1943. Samples in 1948 showed a considerable increase in number and a slight increase in weight. This increase, however, consisted almost entirely of midge-fly larvae (Chironomus sp.). Other important fish-food forms, such as hellgrammites, caddis-fly larvae, and May-fly nymphs, have not yet appeared in any degree of abundance.

Though observations in 1947 did not disclose the presence of minnows in the lower river, in 1948 minnows were observed to be present at Stations 8 and 9 and fairly abundant at Stations 10 and 11.

Although fishing in this stream is still extremely limited, reports from fishermen in the summer of 1948 showed that sunfish, channel catfish, and carp were found in the vicinity of Stations 9 and 10. No reports of bass being caught in this section of stream were received, but several small bass (4 to 5 inches) were observed at Station 10.

A fair number of fishermen were observed at Station 11 during the summer of 1948. Though most fishermen considered fishing very poor, quite a few catches of sunfish, channel catfish, and carp--and even a few bass--were reported.

Though some improvement (both chemical and biological) has been made in the lower river, number and types of bottom animals still show a serious state of depletion. No mollusks and very few of the other major fish-food organisms are present (table 5). Until these bottom-animal conditions improve considerably, and until further field studies (including bottom samples) verify such improvement, it cannot be assumed that this section of the river is free of serious pollution.

CONCLUSIONS

1. Pollution surveys ordinarily contain no measure of fish-food organisms or fish in a stream.
2. Standard physical, chemical, and bacteriological methods used in pollution surveys do not necessarily present the true picture with respect to fish and other aquatic animals.
3. The lower Shanandoah River affords an example

TABLE 5.--Average number and kinds of bottom animals per square foot

Station No.	Aquatic earthworms	May-fly nymphs	Midgefly larvae	Caddis-fly larvae	Beetle larvae	Hellgrammites	Snails	Misc.	Total
	1937 (June to September, 141 samples)[1/]								
9	1.7	46.8	16.4	37.4	14.8	3.8	6.8	4.8	132.5
	1947 (June to October, 5 samples)								
1	1	171	176	458	83	1	4	18	912
2	27	0.4	56	7	3	0.2	21	3	108
3	1	44	56	389	5	2	25	5	528
5	0.5	175	89	217	29	2	104	11	628
6	0.2	137	83	156	73	6	129	5	590
7	0	0	0	0	0	0	0	0	0
8	0	3	59	0.8	6	0.4	0	0.6	70
9	0.6	0	30	0	1	0.6	0	0	32
10	0	0	0.5	0	0	0.5	0	0	1
11	0	0	16	0	2	0	0	1	19
	1948 (June to September, 2 samples)								
1	3.5	196	271	766	87	3	11	24	1,362
2	0	2	349	174	10	1.5	23	1	560
3	0.5	22	192	331	1	2	12	5	566
5	5	269	26	162	26	2.5	186	38	714
6	0.5	473	163	158	41	7.5	77	17	937
7	0	0	0	0	0	0	0	0	0
8	1	1	80	1	2	0	0	0	85
9	4	0	262	1	2	0	0	1	270
10	1	0	185	0	1	0	0	0	187
11	0.5	3	348	8	1	1	0	0	362

[1/] Surber, E. W. (1942).

of a large stream that is relatively free of pollution according to accepted physical, chemical, and bacteriological standards, yet contains virtually no fish or other aquatic animals because of toxic elements from industrial wastes.

4. The Surber bottom sampler is a tool which may be used, in the Shenandoah River and in similar streams, to determine the impact of pollution on fish-food organisms and consequently on fish. By the simple method of using this sampler, the source, degree, and extent of pollution may be determined.

LITERATURE CITED

American Public Health Association
1946. Standard methods of wateranalysis. American Public Health Association, New York, 9th ed., 286 pp.

Beatty, R. O.
1947. Pollution, pollution abatement and the wildlife crop. Izaak Walton League of America, Chicago, Ill., 36 pp.

Davis, H. S.
1938. Instructions for conducting stream and lake surveys. U.S. Bur. Fish., Fish. Circ. 26, 55 pp., illus.

Ellis, M. M.
1937. Detection and measurement of stream pollution. U.S. Bur. Fish. Bull. 22, 72 pp., illus.

1940. Pollution of the Coeur D'Alene River and adjacent waters by mine wastes. U.S. Fish and Wild. Serv., Spec. Sci. Rept. 1, 61 pp.

- - - Westfall, B. A.: and Ellis, M. D.
1946. Determination of water quality. U.S. Fish and Wild. Serv., Res. Rept. 9, 117 pp.

Hart, W.B.; Doudoroff, Peter; and Greenbank, John.
1945. The evaluation of the toxicity of industrial wastes, chemicals and other substances to freshwater fishes. Waste Control Lab., Atlantic Refining Co., Philadelphia, Pa., 317 pp.

Lackey, J. B.
1940. Limitations of Euglenidae as polluted water indicators. Pub. Health Repts. 55 (7): 268-280

McGauhey, P. H.; Eich, H.F.; Jackson, H.W.; and Henderson, Croswell.
1942. A study of the stream pollution problem in the Roanoke, Virginia, metropolitan district. Va. Poly. Inst., Eng. Expt. Sta., Series 51.

Newton, Lilly
1944. Pollution of rivers of West Wales by lead and zinc mine effluent. Annals, Applied Biology 31: 1-11.

Platner, W.S.
1946. Water quality studies of the Mississippi River. U.S. Fish and Wild. Serv. Spec. Sci. Rept. 30, 77 pp.

Purdy, W.C.
1930. A study of the pollution and natural purification of the Illinois River. Pt. 2, The plankton and related organisms. Pub. Health Bull. 198.

Roetman, E. T.
1944. Viscose-rayon manufacturing wastes and their treatment. Water Works and Sewerage, July and August 1944.

Surber, E. W.
1937. Rainbow trout and bottom fauna production in one mile of stream. Trans. Am. Fish. Soc. 66 (1936): 193-202.

1939. A comparison of four eastern smallmouth bass streams. Trans. Am. Fish. Soc. 68 (1938): 322-335.

1942. A quantitative study of the food of the smallmouth black bass (Micropterus dolomieu) in three eastern streams. Trans. Am. Fish. Soc. 70 (1940): 311-334.

Wiebe, A.H.
1928. Biological survey of the upper Mississippi River with special reference to pollution. Bull. U.S. Bur. Fish. 43 (1927) pt. 2: 137-167. (Doc. 1028).

West Virginia Water Commission.
1948. Potomac basin zoning report, July 1, 1948. West Virginia Water Commission, Charleston, W. Va., 40 pp.

Reproduced With Permission From:
SEWAGE AND INDUSTRIAL WASTES
25(1953): 210-217

AQUATIC ORGANISMS AS AN AID IN SOLVING WASTE DISPOSAL PROBLEMS*

By Ruth Patrick

Curator, Dept. of Limnology, Academy of Natural Sciences of Philadelphia, Philadelphia, Pa.

This paper discusses the various ways in which aquatic organisms may be of use in solving problems associated with waste disposal. Since many state and federal laws set forth that nothing may be discharged that is deleterious to aquatic life, the most expedient way to determine the effect of an effluent is to study the aquatic organisms themselves.

In every river that has not been adversely affected by pollution there is a great variety of aquatic life. These organisms do not represent a great mass of living things, but rather they are organized into an intricately balanced system, often referred to as a food chain of biodynamic cycles.

Bases of Food Chain

At the base of the food chain are the bacteria. These organisms use the complex wastes entering a river as a source of energy in their metabolism. In so doing they break down the wastes into substances that can be used as a source of food by other organisms. These processes, which are often referred to as decay or decomposition, occur most rapidly when the bacterial population is of optimum size. When the bacteria become too numerous the processes are slowed down. The protozoa and other small invertebrates which feed on bacteria are instrumental in keeping the bacterial populations in check.

The algae are also at the base of the food chain. They are able to utilize inorganic substances to make proteins and carbohydrates, which are used as a source of food by other organisms. Indeed algae have often been referred to as the grasses of the sea. Upon them not only the many different invertebrates, but also some fish and other vertebrates, feed directly. Besides their value as a source of food they also replenish the oxygen supply of a river by a process known as photosynthesis. This is the method by which carbohydrates are synthesized and oxygen is given off as a by-product. Indeed, in many rivers this is the principal way in which oxygen is restored after it has been depleted.

The algae and the bacteria are the most important organisms in bringing about the "rejuvenation" or "cleansing" of a river. The roll of the fungi is also significant in this respect, but as yet not as well understood.

Many Food Chains Involved

As previously stated, many invertebrates, such as the worms, the snails, and the insects, feed directly on the bacteria, the fungi, and the algae. They in turn are a source of food for the carnivorous species; thus, a closely integrated food chain is formed.

This food chain does not consist, however, of a single series of links, but rather of a series of chains that are sometimes interlinked. Thus, pollution may break one series of links, yet not completely destroy the chain. It is only when pollution is extreme that the chain is completely broken and the higher forms of life are completely eliminated. Thus, when one is concerned with the problems of waste disposal and river conservation, he must concern himself with the whole pattern of life in the river rather than just one group; for example, the fish.

Pollution Effects

There are commonly five ways in which wastes may harm the aquatic life of a river, as follows:

1. They may produce oxygen deficiency. This may be due to the bacteria, which attack the wastes and use oxygen in their metabolic processes. The wastes also may not be completely oxidized when they are discharged and thus take up oxygen from the water in completing the oxidation necessary to stabilize them.
2. They may be toxic to aquatic life. This may be due to the nature of the chemicals themselves. However, it may be due to the pH which they create in the river. Wastes also may be toxic due to the osmotic pressure which they develop in river water, thus bringing about conditions unfavorable for aquatic life.
3. Temperature changes produced by wastes may be harmful in two ways. The amount of change which they produce may be deleterious. It is a well known fact that a sudden change in temperature of more than two degrees is harmful to the sunfish. Also, a waste, by raising or lowering the temperature of a river only two degrees, may cause the temperature of the water to be in a critical range deleterious to the functioning of certain physiological processes necessary for life.
4. The physical properties of the wastes may be harmful. They may carry suspended solids that are abrasive and thus injure mechanically the membrane of the gills of fish. In other cases, such as oil, they may coat the gill structures and thus make the absorption of oxygen from the water impossible.
5. Wastes may render the habitats of aquatic organisms untenable. For instance, suspended solids may settle out and clog up the natural habitats of aquatic organisms. Eggs may become buried. In other

---

*Presented at 25th Annual Meeting, Federation of Sewage and Industrial Wastes Assns.; NewYork, N.Y.; Oct. 6-9, 1952 cases the added pressure created by settleable solids may cause the egg cases to burst. Some wastes produce turbidity, thus hindering light penetration. Thus, the photosynthetic zone of a river will be greatly restricted and the algal production limited.

Besides bringing about death of organisms, waste may lower their resistance to the normal factors in the environment so that eventually the population dies out. To date these effects of wastes have been studied very little.

The Academy of Natural Sciences of Philadelphia has used two approaches to study the effect of pollution on a river - laboratory tests and river surveys.

Laboratory Tests

For determining the oxygen consumption of a waste, a combination of tests are used: immediate oxygen demand, biochemical oxygen demand, and complete oxygen demand. These tests are well described in the literature.

The methods for determining the toxic effects of wastes on aquatic life have, to a great extent, been developed in the Academy laboratory. A considerable part of this work was done with the aid of a grant from the American Petroleum Institute.

Realizing the importance of the bio-dynamic cycle, the effect of a given waste is determined by using organisms representing three stages in the cycle. These organisms are as follows:

1. An alga that is important as a producer of oxygen, and as an organism that can convert inorganic substances into a direct source of food for many aquatic animals.
2. An invertebrate that serves as a direct food for fish. As representatives of this group, insects and snails have been used.
3. Fish, because of their recreational and economic importance.

The fish tests are conducted according to the methodology set forth by the Federation's Subcommittee on Toxicity (1).

Insect and Snail Tests

The insect and snail tests have been patterned after the fish tests. As with the fish, care is taken to assure that the organisms are thoroughly acclimated to laboratory conditions. This is determined by a very low death rate and by the fact that growth is taking place in the acclimatization tank over a period of time. This takes several weeks, and sometimes months, to ascertain. The invertebrate tests are conducted under constant temperature and dissolved oxygen conditions. A constant volume of fluid to organism is maintained. The organisms are not fed during the test. As in fish, death is a difficult condition to establish. It is defined as lack of response to tactile stimulus and failure to recover. This is accompanied by various changes in the appearance of the organisms. In insects the same procedures as those used with fish are followed. In the case of snails, after they fail to respond to tactile stimuli, they are placed in uncontaminated water in which they have been reared. If they do not recover in 48 hr., they are determined to be dead.

Algae Tests

The algae test, although similar fundamentally, are quite different from the fish or insect tests. For these tests the diatom *Nitzschia linearis* was chosen. This diatom is commonly found in eutrophic streams and rivers which have not been adversely affected by pollution in the eastern and midwestern sections of the United States. The tests are conducted in Erlenmeyer flasks. The light source is artificial, being a combination of neon and "daylight" fluorescent lights. The tests are usually conducted at 18° to 20° C., depending on the temperature of the water into which the waste being tested will be discharged. The dilution water, as in the case of the fish, is a natural water or a synthetic water, which has been selected because it matches in chemical composition the water of the river into which the waste will be discharged.

The diatom cultures used in these tests consist of a single species of algae. They are cultured in the laboratory several months before testing, and are known to be maintaining a division rate characteristic of healthy diatoms of this species. Since death is a difficult thing to determine in a diatom, the point at which the growth rate is decreased 50 per cent below that of the control is taken as comparable with the median tolerance limit obtained in fish tests.

In the course of experimentation it has been found that the rate of growth is influenced by the size of the inoculum. Therefore, it is necessary that the same size inoculum be used in the control as in the tests. This is verified by counting the number of cells per milliliter in each flask at the beginning of the experiment. All tests, as well as the control, are run in duplicate. All subsequent counts are made in the same manner as at the beginning of the test to determine the rate of growth.

The duration of the test should be from 5 to 7 days. Often, at the beginning of an experiment, there is a "lag" effect before the diatoms respond to the test medium. This effect may last for 48 hr. From the third to the seventh day is the time when the growth rate can be most accurately correlated with the effects of the test medium. After this length of time some of the necessary nutrients in the dilution water may be used up and the effect produced may be due to malnutrition rather than to toxicity.

The tests described are acute toxicity tests. It is hoped that chronic toxicity tests may be developed in the near future. This would help to determine whether a substance would lower the resistance of an organism so that it could not successfully compete in nature.

Value of Laboratory Toxicity Tests

The tests described would be of value to industry in solving the following types of problems.

1. In the planning of waste disposal, (a) to determine just how much of each type of waste can be safely discharged into a river and (b) to separate the unharmful from the toxic wastes and thus reduce the cost of waste treatment.
2. In changing a process, to determine whether a new process will produce a more severe waste problem.
3. In installing new types of waste treatment, to determine whether the effluent from such a treatment is as harmless as the specifications state.
4. When dumping settling basins at high river flow, to determine how much can be dumped at a given flow without damaging the aquatic life.
5. When an industry is accused of causing a given damage and there are many other effluents emptying into the river, to determine whether or not the accused industry is to blame.

River Surveys

The second approach to solving waste effluent problems is the biological survey of the river. As every aquatic biologist knows, the ecology of the river is a very complex result of many interacting factors. Because of this, no series of toxicity tests can accurately determine the effect of a waste in a river. They merely provide an approximation of what will happen. The only way to know the effect of a waste on a river is to study the river itself.

The methodology for conducting a biological survey was published in the Proceedings of the Academy of Natural Sciences of Philadelphia in 1949. In a river survey, all of the organisms established in a given region of the river are identified as to species. The chemical characteristics of the water are determined. A total bacterial count and a coliform count are made, and the B.O.D.'s are determined.

A histogram is made of each region studied. The heights of the columns are determined by the number of species of each group of organisms living in that part of the river. Since the various groups vary greatly as to the number of species in them, the height of a given column is expressed as a percentage of the number of species of that group found in a river not adversely affected by pollution. By this method the various columns are comparable.

From the pattern developed by the columns of a histogram, the state of "health" of a river is determined. Research makes it evident that the pattern of life based on all groups of organisms is a more reliable criterion for judging the "health" of a river than a single group of "indicator organisms." Just as in other scientific work, the more different evidence available to support conclusions, the more valid they usually are.

Value of River Surveys

One of the great values of this type of study is that it tells the condition in the river over a period of time. Because these aquatic organisms have life histories of varying lengths, one is able by examining the structure of the population to determine when in the past a deleterious effect occurred. This effect can be picked up over a period of a year and sometimes longer. It depends, of course, on the kind and duration of the pollution.

This type of river study may be of use to the industrialist in the following ways:

1. Such a survey before an industry starts to operate will define the condition of the river at that time. There are few large rivers in the eastern part of the United States which have not to some degree been adversely affected by pollution. It is well for the state authorities, as well as the industry, to know what the condition of the river is before the industry starts to operate.
2. This method is useful in determining whether a waste treatment program is sufficient to protect the river, or if more treatment is needed.
3. If an industry is accused of damaging a river, such a survey, comparing various sections of a river, can tell if the complaint is justified.

Such a survey is certainly the most direct approach to use in determining the condition of the river. It is believed that by the previously described toxicity tests and biological surveys definite methods have been developed which should be of great aid to industries and to states in defining their pollution problems.

Reference

1. Doudoroff, P., et al., "Bio-Assay Methods for the Evaluation of Acute Toxicity of Industrial Wastes to Fish". THIS JOURNAL, 23, 11, 1380 (Nov., 1951).

DISCUSSION

By Arden R. Gaufin and Clarence M. Tarzwell

Limnologist and Chief, Biology Section, respectively, USPHS Environmental Health Center, Cincinnati, Ohio

Cleaning up the rivers, lakes, and bays of the country will require a great deal of money and the cooperative effort of many different groups of people. To accomplish this task and properly control the disposal of industrial and municipal wastes into surface waters, the pollutional nature of these wastes and their influence on aquatic life must be considered.

The value of fishery resources and the magnitude of the economic loss caused by the destruction of aquatic life by the industrial and municipal pollution of waters are being more widely recognized. Many states have adopted legal measures providing for the protection of fish and other aquatic life from pollution. While some have interpreted this legislation as applying only to the acute poisoning or killing of fish, Dr. Patrick's group has dealt with the fish food organisms, as well as the fish, and also has given consideration to the chronic effects of wastes on aquatic life.

In studying the effect of pollution on a river, the best type of biological program is that which recognizes the complexity of the ecological factors involved. In combining laboratory tests with river surveys, the author is attempting to gather as many different types of evidence as possible before drawing any conclusions. She is to be commended for using an approach which is more thorough than that normally used in the past for examination of this complex problem.

The need for experimental studies dealing with the toxicity of pollutants to aquatic life is great. Dr. Patrick has already discussed some of the methods and the importance of conducting such toxicity tests. Fish bio-assay procedures for most industrial wastes are not costly and are not especially difficult to perform. On the basis of toxicity determinations, it is usually possible to predict whether a waste can be discharged at a given rate without causing direct injury to fish in the receiving water. Such data also are helpful in determining the amount of treatment required, the portion of the waste requiring treatment and the effectiveness of treatment methods (1).

It has been mentioned by Dr. Patrick that another use of this method is to determine whether the discharges of a given industry are responsible for causing damage when there are many effluents emptying into the river. In such a case the character of the receiving stream is of considerable importance in determining the toxicity of a waste. Further, the toxicity of wastes can be greatly influenced by interactions between their individual components and the dissolved minerals present in widely varying amounts in receiving waters. For instance, the salts of heavy metals are generally more toxic in soft or acid waters than they are in alkaline water. Synergy and antagonism must be considered. For example, mixed solutions of cupric and zinc salts have been found to be much more toxic to minnows, than either metallic salt alone (2).

Dr. Patrick's method for conducting a biological survey of a river is to be commended for the completeness of its scope and for attempting to formulate criteria which might be useful in evaluating the effects of pollution on streams. However, for many purposes it should not be necessary to conduct such extensive or complicated studies as those outlined.

The concept of a healthy stream as being one with a large number and wide variety of species may serve as an index of conditions in some streams, but there are many areas in which it will not apply. For example, in many of the purest streams the variety and abundance of both fish and invertebrate life is distinctly limited. In Colorado and Utah many trout streams have a fish fauna of as few as 3 to 5 species and the variety and abundance of bottom fauna depends largely on the geological nature of the drainage basin (3) (4). The water in these streams is clear, sparkling, and usually meets drinking-water standards. These streams are not biologically abnormal or polluted from any standpoint.

Although heavy pollution drastically reduces the number and variety of species in a stream, limited organic pollution may fertilize a stream and increase production. There is also a great increase in the varieties of aquatic life following recovery in streams polluted with many organic wastes. Many polluted streams have a greater number of fish species than do the purest streams. Indices of stream conditions developed in a local area should be applied only in those areas having similar ecological characteristics.

In stream sanitation work it is not essential that the biodynamic cycle be preserved in its primitive condition. Such conditions have already been largely eliminated by deforestation, overgrazing, mining, and agricultural practices. The objective now is to manage waters so that they will produce the maximum sustained yield of recreation and sport and commercial fishing consistent with the capacity and other reasonable uses of the waters. Among the aquatic fresh-water organisms, fish are the most important to the general public. The destruction of a few sensitive species is of little importance if they are replaced by others equally desirable so that the fish yield is not impaired. In the final analysis the fish yield is the important measure of effective stream management and fishlife should be considered as the major index of stream conditions.

Dr. Patrick's system for making stream surveys is costly and requires the help of a considerable number of well-trained scientists for it to be usuable. Many state agencies charged with water pollution control, as well as small industries, do not have money or personnel for a biological program of the magnitude recommended.

When conducting biological investigations for the evaluation or solution of pollution problems, careful formulation of objectives is required. If the objective is to determine only general stream conditions, a reconnaissance survey is favored to determine the relative quantitative and qualitative aspects of the biota. In such a program the pollutional condition of a stream can often be determined by reference to those groups of organisms which best reflect the ecological conditions under which they live. If the objective of a stream survey is to ascertain the economic loss caused by the damaging effects of pollutants on the fishery of the stream, then it is necessary to determine the composition of the fish fauna in the stream and the changes in that fauna which might have occurred in the past. Necessary data on fish populations and yield can be obtained by creel censuses, records of commercial catches, seining, gill netting, trapping, etc. Since the procedures for conducting fishery yield surveys have been fairly well standardized by workers in fish management, it is not deemed advisable to dwell further on the subject here.

Several different approaches have been advocated by biologists in using aquatic organisms as indicators of the pollutional conditions of a stream. Dr. Patrick, emphasizing primarily a qualitative approach, maintains that the total number of species, rather than the qualitative and quantitative characteristics of the population, constitutes the most valuable index as to the health of a stream. Ellis (5) advocated a semi-quantitative approach when he stated that the relative abundance of indicator species was the important consideration. Biologists of the USPHS Environmental Health Center at Cincinnati, Ohio, have found that both criteria are important and serve best when used concurrently. For example, Gaufin and Tarzwell (6) found that in a small polluted stream near Cincinnati, the biota in the polluted zones was characterized by few species but large numbers of individuals, whereas in the clean-water zones there were many species but comparatively few individuals of each species.

Quantitative measurements of the total number of species or individual organisms in any given area of a stream are often difficult to obtain. For example, Environmental Health Center biologists took a series of nine random samples, by means of an Ekman dredge, from a pool in a small sewage polluted stream near Cincinnati. A total of 50 species of macro-invertebrates was collected. On the average, it was determined that any three of these samples would have yielded only 60 per cent of the 50 species. Seven samples would have been required to have obtained 90 per cent of the types represented.

Where personnel are not available to do all of the technical taxonomic work required for species identification, or to take enough quantitative samples to accurately determine the abundance of individual species or organisms, a practical biological inventory is still possible.

Specifically, the degree and extent of pollution in a stream can be determined accurately by reference to the macro-invertebrate fauna, particularly that found in the riffles. A biological analysis of the pollutional status of a stream can be obtained in the field through recognition of the biological orders, families, or genera in the invertebrate associations encountered. This type of biological inventory is superior to limited chemical data, as the complex of such organisms which develops in a given area is in turn indicative of present, as well as past, environmental conditions in that area. Bottom organisms are more fixed in their habitat than are fish or plankton and cannot move to more favorable surroundings when pollutional conditions are most critical.

Shortened procedures, such as that suggested, cannot be recommended for use by anyone except a well-trained aquatic biologist. When used properly, however, such techniques can be of considerable value to organizations having waste disposal problems to solve.

References

1. Doudoroff, P., "Biological Observations and Toxicity Bio-Assays in the Control of Industrial Waste Disposal." Proc. 6th Ind. Waste Conf., Purdue Univ. (1951).
2. Doudoroff, P., "Some Recent Developments in the Study of Toxic Industrial Wastes." Proc. 4th Pacific Northwest Ind. Waste Conf., Washington State College (1952).
3. Gaufin, A.R., "A Comparative Study of the Bottom Fauna Productivity of the North and South Forks of the Provo River at Stewart's Ranch, Utah." Midwest Wildlife Conf., University of Wisconsin (In manuscript form) (1949).
4. Pennak, R.W., and Van Gerpen, E.D., "Bottom Fauna Production and Physical Nature of the Substrate in a Northern Colorado Trout Stream." Ecology 28, 1 (1947).
5. Ellis, M.M., "Detection and Measurement of Stream Pollution." U.S. Bureau of Fisheries, Bull. 22 (1937).
6. Gaufin, A.R., and Tarzwell, C. M., "Aquatic Invertebrates as Indicators of Stream Pollution." Pub. Health Rep., 67, 57 (1952).

DISCUSSION

By Ruth Patrick

As Dr. Gaufin has pointed out, there may be a synergistic effect between an effluent entering a river and substances already in a river. For this reason toxicity tests may be used as a yardstick, but one must study the river itself to determine accurately the effect of an effluent.

It is true that there are many different types of rivers in the country, with varying amounts of aquatic life. However, the writer has yet to find a river with two ecologically similar areas; one adversely affected by pollution with industrial or municipal wastes, and one unpolluted in which the unpolluted area did not have a greater diversity of species of diatoms, insects, and fish than the polluted area.

It is correct that a well-qualified biologist can determine that a river is badly polluted without determining all the species. But if it is desired to determine trends of conditions or have definite evidence for future comparison, the species present must be determined.

Very rarely are all the species of a genus indicators of pollution. For this reason, it would be very dangerous to draw positive conclusions from determination only to genus.

Dr. Gaufin indicated that the method described is a qualitative one. It is qualitative in that the kinds of species composing the biodynamic cycle are considered. It is, however, quantitative in that the measure also considers the number of species. No one has yet devised a statistically valid quantitative method for benthic forms in a river based upon the number of individuals.

Reproduced With Permission From

THE PROGRESSIVE FISH-CULTURIST
17(1955): 64-70

EFFECTS OF SILTATION, RESULTING FROM IMPROPER LOGGING, ON THE BOTTOM FAUNA OF A SMALL TROUT STREAM IN THE SOUTHERN APPALACHIANS

L. B. Tebo, Jr.
North Carolina Wildlife Resources Commission
Hoffman, North Carolina

SILTATION, RESULTING FROM IMPROPER LAND - USE PRACTICES, is regarded as one of the most important factors contributing to a reduction in the acreage of desirable fishing waters in the United States. Although much information of a general nature has been published, there is a lack of quantitative data regarding the effects of siltation on stream values.

One phase of a Dingell-Johnson project, established by the North Caroline Wildlife Resources Commission during the summer of 1952, was to obtain quantitative data regarding the effect of siltation on trout streams in the southern Appalachians. The project was begun on the Coweeta Experimental Forest, located in Macon County, North Carolina, where for 20 years the U. S. Forest Service has been collecting extensive data regarding the effects of various land use practices on experimental watersheds. The purpose of this report is to present data regarding the effects of siltation on the bottom organisms of Shope Creek, a small trout stream which received the drainage from a 212-acre logged watershed (figure 1).

During 1942, logging was commenced on the 212-acre watershed on the Coweeta Experimental Forest. The periods of activity on the watershed were:

May 1942 - Mar. 1943: Active logging
Mar. 1943 - Jan. 1945: No logging
Jan. 1945 - Nov. 1948: Active logging
Nov. 1948 - Apr. 1953: No logging
Apr. 1953 - Present: Active logging

The logging was carried out by local contractors, with no limitation of methods or supervision by the Forest Service. Logs were ground-skidded by teams. Because of steep slopes, the roads and skid trails were built parallel and adjacent to the channel of the drainage stream. The roads were characterized by excessively steep grades alternating with level stretches. No surfacing material and no drains or water cutoffs were used on the roads. With the termination of the original logging in 1948, 2.2 miles of road had been constructed on the 212-acre watershed.

Description of Shope Creek

Shope Creek, which received the stream from the logged watershed, flows into Coweeta Creek and thence to the Little Tennessee River. Shope Creek drains a watershed of approximately 1,880 acres and is typical of many smaller trout streams in the southern Appalachians. Average monthly streamflow for a 6-year period ranged from a low of 2.31 c.f.s. during October to 8.32 c.f.s. during February (figure 2). Figure 3 illustrates the frequent occurrence and magnitude of floods occurring in this small trout stream.

During the period of this study, stream temperatures have ranged from a low of 33.0° F. during December 1952 to a high of 65.5° F. during August 1953. During the fall of 1953, the water had a pH of 6.6 and a methyl orange alkalinity of 8.0 p.p.m.

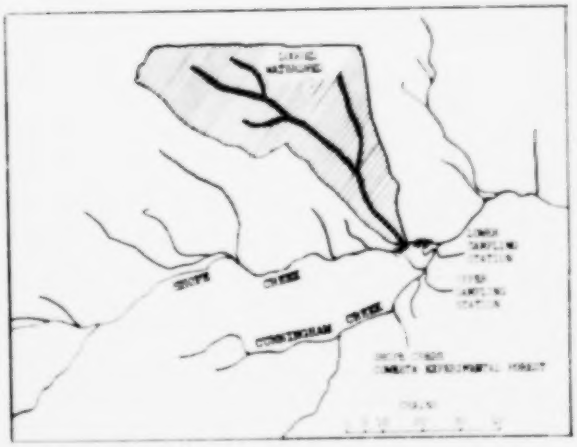
The upper portion of the stream is characterized by steep gradient (900 feet to the mile) with series of cascades and low waterfalls, interspersed with large pools having excellent shelter in the form of large boulders and broken water surface. The bottom is predominantly boulders and rubble with occasional outcrops of granite bedrock. From approximately one-fourth mile above the sampling stations to the lower boundary of the experimental forest, there is a noticeable change in the habitat. The gradient is 224 feet per mile, and the cascades and waterfalls of the upper section are replaced with short riffles and shallow pools. There is no rooted aquatic vegetation in the stream.

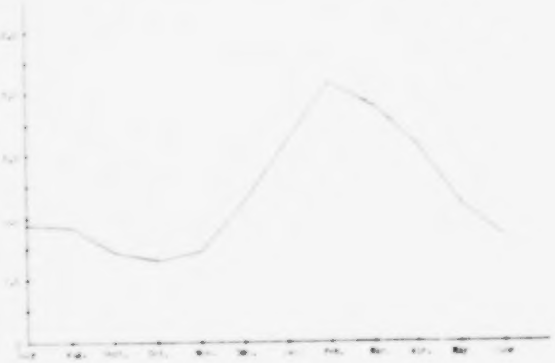
As nearly as can be determined, no trout have been stocked in Shope Creek since 1930, when rainbow trout were introduced by local residents. At present, the upper and lower reaches of the stream contain brook and rainbow trout, respectively, with an intermingling of these two species in the section just above the mouth of the stream from the logged watershed. No fishing has been permitted for the past 4 or 5 years. However, prior to closure, the stream had an excellent reputation among local fishermen.

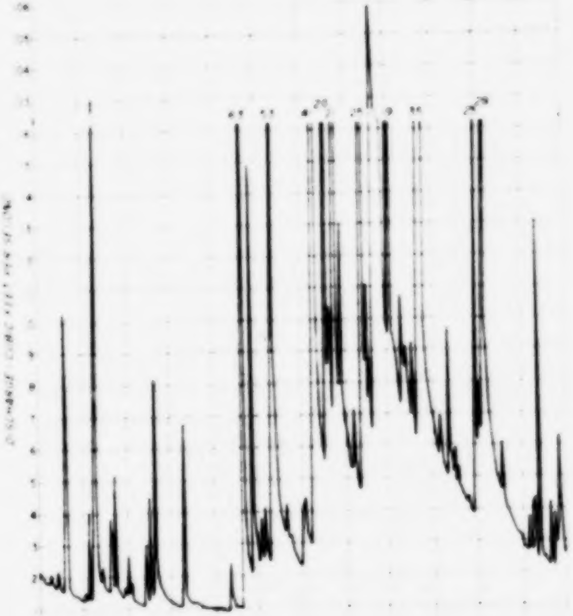
Water Quality

During storm periods, the effect of the stream from the logged watershed (Watershed No. 10) on Shope Creek is illustrated by the turbidity of water samples collected at the mouth of the stream from No. 10, from Shope Creek above the mouth of No. 10, and from Shope Creek below the mouth of No. 10.

Presented at the Eighth Annual Conference of the Southeastern Association of Game and Fish Commissioners, New Orleans, Louisiana, November 1-3, 1954, to report on one phase of a Dingell-Johnson project undertaken by the U.S. Fish and Wildlife Service, U.S. Forest Service, and North Carolina Wildlife Resources Commission.







Date	Turbidity (p.p.m.)		
	Stream from Number 10	Shope Creek Above 10	Shope Creek Below 10
Apr. 11, 1947 ---	1,200	25	390
Feb. 20, 1954 ---	1,371	67	261

The roads and skid trails proved to be the major source of turbidity (Lieberman and Hoover 1948). Skidding logs down the steep slopes creates channels which concentrate runoff, resulting in a high rate of erosion. For the 2-year period from April 1951 to March 1953, an average of 5.34 cubic feet of soil per lineal foot of road surface were eroded from the logging road. This would amount to a loss of 2,297 cubic yards of soil for the total 2.2 miles of road system.

During periods of low streamflow, the physical effects of siltation on Shope Creek are noticeably evident. During the low flows of late summer and fall, the bottom of Shope Creek above the mouth of the logged watershed accumulates a thin layer of finely divided organic matter, while below the mouth of the logged watershed the stream bottom in both pools and riffles is covered with a layer of sterile sand and micaceous material which may accumulate to a measured depth of 10 inches.

Bottom Fauna

Because of its relative stability in location, the bottom fauna was selected to obtain a measure of the effects of siltation on the stream community. The limited section of Shope Creek affected by siltation from the logged watershed made a direct evaluation of the fish population impractical. The small stream from the logged watershed is too small to support a resident trout population.

Within limits of space and reproductive capacity, the available food in a stream can certainly be regarded as a factor limiting the production of trout. Leonard (1948) and Henry (1949) have stated that in Michigan trout waters the food supply often is the most important limiting factor in trout production. Allen (1951), working on New Zealand streams, found that the bottom fauna was a limiting factor in the production of brown trout. Tarzwell (1938b) found an apparent relation between the quantities of stream foods present and trout production in streams in the southwestern United States.

(Top to Bottom)

FIGURE 1. -- Map of Shope Creek, Coweeta Experimental Forest, showing the location of logged area and sampling stations.

FIGURE 2. -- Mean monthly streamflow (c.f.s.) of Shope Creek for the 6-year period, 1937-42.

FIGURE 3. -- Peak daily streamflow (c.f.s.) of Shope Creek for the period, July 1952 to June 1953.

(Drawings courtesy of the author)

In trout streams of western North Carolina the food of trout is obtained from three sources: the bottom fauna, terrestrial insects, and fish. Analysis of 241 rainbow trout stomachs collected from streams of western North Carolina during 1952 and 1953 indicate that, from January to June, 83 percent of the diet is obtained from the bottom fauna. From June to December, 42 percent of the food of rainbow trout is obtained from the bottom fauna. Terrestrial insects are of major importance during the summer and fall months. Of the 241 trout stomachs examined, only 1 specimen contained fish remains and 3 had eaten salamanders.

From October 1952 to June 1953, 108 square-foot bottom samples were collected at monthly intervals from Shope Creek immediately above and below the mouth of the stream draining the logged watershed. The standing crop of bottom organisms was at all times very low, with a high average of 49.0 organisms per square foot occurring at the untreated station on November 13 (table 1). The highest average volume occurred in the samples of January 14 at the untreated station. The high volume occurring on January 14 resulted from an abundance of large cranefly larvae, *Tipula* sp., and the stonefly nymph, *Pteronarcys scotti*. The frequent occurrence of floods (figure 3) is undoubtedly an important factor contributing to the low quantities of bottom fauna produced in this small trout stream.

From October 1952 to February 1953, the upper station had a significantly larger numerical standing crop of bottom organisms than did the lower station, which was subjected to the siltation from the logged watershed (tables 2 and 3). The volume of bottom organisms was greater in the control section on all but two sampling dates, April 23 and May 21, 1953 (table 1).

A major flood that occurred on February 21, 1953, increased the flow in Shope Creek from 6.7 c.f.s. to 105.8 c.f.s. in a 24-hour period (figure 3). The flood completely resorted bottom materials and flushed the deposited sediments downstream, exposing the original rubble and gravel bottom. On February 26, 1953, the numbers of bottom organisms at the lower station had been reduced 73.2 percent, as compared with the January level, while the numbers at the untreated station had been reduced 22.2 percent (table 1).

High water levels plus frequent rains February to May (figure 3) prevented a reaccumulation of silt in the lower section of Shope Creek. On April 2, April 23, and May 21, that section of stream produced slightly greater standing crops of bottom organisms than did the control section. The difference was not significant (F = 0.208 d.f. = 1 and 30), and was the result of an increase in the numbers of mayfly nymphs in the treated section of stream (table 1). The inexplicable superiority of mayflies in the treated section of stream may have been the result of reduced competition and improvement in habitat, both caused by the February flood.

When samples were collected in June 1953, silt and sand again had begun to accumulate in the treated section of the stream, and the control section again produced a greater average standing crop of bottom organisms (table 1). The difference was not statistically significant (t = 1.42 d.f. = 10).

Before the reduction in the quantity of stream bottom organisms, from October through February, can be attributed to the effects of siltation, it is necessary to assume that there was no difference between the two sampled stations prior to logging. The study was commenced quite some time after logging took place, and it is therefore impossible to test this basic assumption. However, the fact that the sampled areas are on immediately adjacent and similar sections of the same stream, as well as the comparable quantities of bottom fauna produced during the spring months, when silt did not accumulate in the treated section of the stream, lends support to the assumption that there were no pretreatment differences between the two stations sampled.

With the exception of the difference in mayflies during the spring months, as noted above, there were no appreciable qualitative differences between the two stations sampled (table 1).

Discussion

The period during which the standing crop of organisms in the treated section of Shope Creek was significantly lower than in the control section coincided with the period of maximum accumulation of inorganic silt and sand. Inorganic silt and sand have poor ability to support a fauna. Tarzwell (1938a) found that mineral silt bottoms were poor in food. Murray (1938) stated that, in Indiana streams, sand by itself is likely to be barren of life.

In addition to its poor ability to support a fauna, the shifting sand created an unstable habitat, and organisms inhabiting it were particularly vulnerable to decimation by flood waters. The flood during February removed the accumulated sediments and resulted in a drastic reduction in the number and volume of bottom organisms in the treated section of stream. During the high flows and frequent rains from February to May, the rate of dilution by clear water from the main fork of Shope Creek prevented the reaccumulation of sediment in the treated section of stream. A fauna which resulted was quantitatively comparable to that found in the control section. It is doubtful that the rapid recovery after the flood -- undoubtedly by means of the drift of organisms from the control section -- could occur if all of the Shope Creek watershed were subject to the effects of siltation.

The low fertility and frequent occurrence of floods in western North Carolina trout streams results in a low production of stream bottom organisms under the very best conditions. Therefore, because of the dependence of trout on stream-produced organisms, any outside factor, such as siltation, which reduces the normally low quantities of stream organisms will ultimately have a deleterious effect on the trout population.

It is apparent from the Coweeta studies that poorly planned road systems and the promiscuous use of smaller stream channels as skid trails result in a

TABLE 1.--Numbers and volume of bottom organisms collected from riffles in Shope Creek from October 1952 to June 1953 at stations above and below the mouth of a tributary stream draining a logged watershed

	October 16		November 13		December 17		January 14		February 26	
	Above	Below	Above	Below	Above	Below	Above	Below	Above	Below
Number of samples	3	3	3	3	6	6	6	6	6	6
Total number of organisms	116	74	147	78	226	137	234	164	182	44
Number per square foot	38.7	24.7	49.0	26.0	37.5	22.8	39.0	27.3	30.3	7.3
Standard deviation[1]	9.29	13.0	12.1	14.7	13.6	6.80	15.4	16.9	22.3	3.19
Total volume (cc)[1]	0.70	0.15	0.70	0.20	3.20	0.50	5.70	2.40	2.65	trace
Volume per square foot	0.23	0.05	0.23	0.07	0.53	0.08	0.95	0.40	0.44	trace
Diptera	27	4	57	33	65	55	90	70	76	13
Trichoptera	18	12	7	5	25	20	19	9	10	4
Plecoptera	25	9	49	------	57	24	50	18	9	6
Ephemeroptera	30	23	22	29	53	23	54	51	36	16
Odonata	1	------	------	------	2	------	2	1	1	------
Coleoptera	11	25	12	11	23	15	19	15	21	5
Oligochaeta	4	1	------	------	------	------	------	------	------	------

	April 2		April 23		May 21		June 12	
	Above	Below	Above	Below	Above	Below	Above	Below
Number of samples	6	6	6	6	6	6	6	6
Total number of organisms	259	283	239	249	180	196	256	202
Number per square foot	43.2	47.2	39.8	41.5	30.0	32.7	42.7	33.7
Standard deviation[1]	17.6	17.6	19.3	26.6	9.27	15.2	11.3	10.7
Total volume (cc)[1]	5.20	3.75	2.50	2.70	1.35	2.60	3.80	1.20
Volume per square foot	0.87	0.63	0.42	0.45	0.23	0.43	0.63	0.20
Diptera	41	33	32	24	25	16	9	10
Trichoptera	13	11	8	5	4	11	18	16
Plecoptera	41	29	24	20	17	31	75	21
Ephemeroptera	148	190	160	189	106	130	112	127
Odonata	------	1	------	------	------	------	2	------
Coleoptera	15	17	12	10	25	5	29	24
Oligochaeta	------	------	1	1	2	2	4	1
Crayfish	1	1	1	------	1	1	3	1
Salamanders	------	1	------	------	------	------	------	------

---

[1] Does not include salamanders and crayfish.

TABLE 2.--Analysis of variance on the basis of total numbers of organisms in October and November 1952

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Between stations--------	1	1,027	1,027	[1]6.62
Between months----------	1	102	102	------
Interaction-------------	1	62	62	------
Error-------------------	8	1,239	155	------

[1] Significant at 5-percent level.

TABLE 3.--Analysis of variance on the basis of total numbers of organisms in December 1952 and January and February 1953

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Between stations--------	1	2,434	2,434	[1]15.02
Between months----------	2	1,372	686	4.23
Interaction-------------	2	207	104	------
Error-------------------	29	4,706	162	------

[1] Significant at 1-percent level.

high rate of erosion and consequent siltation of the stream channel. Steep grades, lack of allowance for proper drainage, and the proximity of roads to stream channels are particularly conducive to siltation. Also, it is the opinion of many foresters that properly constructed roads, in addition to conserving water values, will, in the long run, pay the logging operator by reducing road maintenance work. Where important fishery values are involved, it is imperative that skid trails and road systems be carefully located and constructed.

Summary

1. From 1942 to 1948, a 212-acre watershed on the Coweeta Experimental Forest, Macon County, North Carolina, was logged by a local contractor. Roads and skid trails were built parallel and adjacent to the stream channel. No surfacing material and no drains were used.

2. The physical and chemical characteristics of Shope Creek, a small trout stream which receives the stream from the logged watershed, are described.

3. During storm periods the turbidity of Shope Creek was appreciably increased by the highly turbid waters from the logged area. The accumulation of sand and silt in Shope Creek below the mouth of the stream from the logged watershed is described.

4. Roads and skid trails proved to be the major source of turbidity. From April 1951 to March 1953, an average of 5.34 cubic feet of soil per lineal foot of road surface was eroded from the logging road.

5. From October 1952 to June 1953, 108 square-foot bottom samples were collected at monthly intervals in Shope Creek at stations above and below the mouth of the stream from the logged watershed.

6. From October 1952 through January 1953, the period of maximum accumulation of sediment in the affected section of Shope Creek, there was a significantly lower standing crop of bottom organisms at the station below the mouth of the logged watershed.

7. A flood on February 21, 1953, removed the accumulation of sand and silt in Shope Creek below the mouth of the logged watershed and reduced the bottom fauna at the lower station to 7.3 organisms per square foot, as compared with 25.5 organisms per square foot at the upper station, which had not been subject to siltation from the logged watershed.

8. The February flood exposed an excellent bottom of rubble and gravel at the lower station; from February through May spring rains and high streamflow prevented the reaccumulation of sand and silt at the lower station on Shope Creek. During this period there was no significant difference in the standing crop of bottom fauna at the control and treated stations. During June, when silt had begun to reaccumulate, the control section again produced a larger standing crop of bottom organisms. The difference was not statistically significant.

Acknowledgments

Facilities of the U.S. Forest Service station at Coweeta Hydrologic Laboratory have been utilized freely in the conduct of this study. The cooperation and advice of Mr. E.A. Johnson and Dr. T. C. Nelson, technicians at Coweeta, have been particularly helpful.

Mr. J. L. Kovner gave advice regarding the statistical analysis of data, and his assistance is gratefully acknowledged.

The author is particularly indebted to Mr. J. H. Cornell, Chief, and to Mr. Duane Raver, Federal Aid Coordinator, Fish Division, North Carolina Wildlife Resources Commission, who were in immediate supervision of the project and whose efforts made the work possible.

Literature Cited

Allen, K. R.
1951. The Horokiwi stream: A study of a trout population. New Zealand Mar. Dept., Fish Bull. 10, 231 pp., illus.

Henry, K. W.
1949. Michigan trout waters. Mich. Forester 30: 13-15, 41.

Leonard, J. W.
1948. Importance of fish food insects in trout management. Mich. Cons. 17 (1): 8-9.

Liberman, J. A. and M. D. Hoover.
1948. The effect of uncontrolled logging on stream turbidity. Water and Sewage Works 95 (7): 255-258.

Murray, M. J.
1938 An ecological study of the invertebrate fauna of some northern Indiana streams Invest. Ind. Lakes and Streams 4 (8): 101-110.

Tarzwell, C. M.
1938a. Factors influencing fish food and fish production in southwestern streams. Trans. Am. Fish. Soc. 67 (1937): 246-255.

1938b. An evaluation of the methods and results of stream improvement in the Southwest. Trans. N. Am. Wildl. Cong. 3: 339-364.

Reproduced from PUBLIC WORKS, 90 (1959): 104-110

STREAM LIFE AND THE POLLUTION ENVIRONMENT*

Alfred F. Bartsch
and
William Marcus Ingram

Increased field investigations over the past 10 years, directed toward the abatement of pollution, have prompted this pictorial presentation to show the impact of pollution upon the stream environment and in turn upon the stream life, or biota. The illustrations were developed initially for use in training sanitary engineers and supporting scientists at the U. S. Public Health Service's Robert A. Taft Sanitary Engineering Center in Cincinnati, Ohio.

To show schematically the effects of pollution on biota, raw domestic sewage has been chosen as the pollutant. With such a waste, the lowering of dissolved oxygen and formation of sludge deposits are the most commonly seen of the environmental alterations that damage aquatic biota. Fish and the organisms they feed on may be replaced by a dominating horde of animals such as mosquito wrigglers, bloodworms, sludge worms, rattailed maggots and leeches. Black-colored gelatinous algae may cover the sludge and, as both rot, foul odors emerge from the water and paint on nearby houses may be discolored. Such an assemblage of abnormal stream life urges communities not to condone or ignore pollution, but to abate it without delay. This biotic picture emphasizes that pollution is just as effective as drought in reducing the utility of a valuable water resource. They help to make clear that pollution abatement is a vital key to the over-all problem of augmenting and conserving waters of this land.

No two streams are ever exactly alike. In their individualism streams differ from each other in the details of response to the indignity of pollution. In the following paragraphs, and in the charts they describe, the hypothetical stream is made to conform exactly to theory, showing precisely how an idealized stream and its biota should react in a perfect system. In reality, of course, no stream will be exactly like this although the principles shown can be applied with judgment to actual problems that may be encountered.

ASSUMED CONDITIONS

The stage for discussion is set in Figure 1. The horizontal axis represents the direction and distance of flow of the stream from left to right. Time and distance of flow downstream are shown in days and also in miles. The vertical scale of quantity - or more accurately, concentration - expressed in parts per million, applies to dissolved oxygen and biochemical oxygen demand at distances upstream and downstream from the origin of the sewage discharge, which is identified as point zero. Here, raw domestic sewage from a sewered community of 40,000 people flows to the stream. The volume flow in the stream is 100 cubic feet per second, complete mixing is assumed, and the water temperature is 25°C. Under these conditions the dissolved oxygen (D.O.) sag curve reaches a low point after two and one-quarter days of flow and then

*Originally published with colored illustrations. Editorial changes have been made to make this text conform with the halftone illustrations.

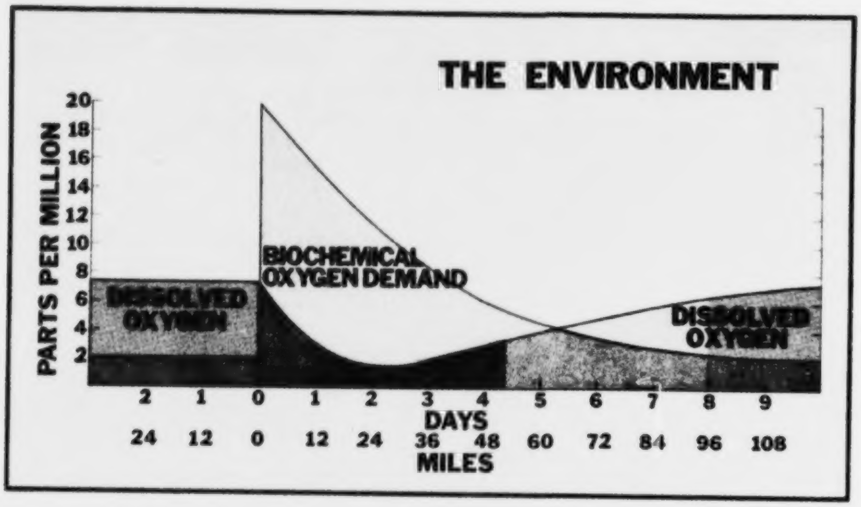


Figure 1 - The assumptions in the hypothetical pollution case under discussion are a stream flow of 100 cfs, a discharge of raw sewage from a community of 40,000 and a water temperature of 25°C, with typical variation of dissolved oxygen and BOD.

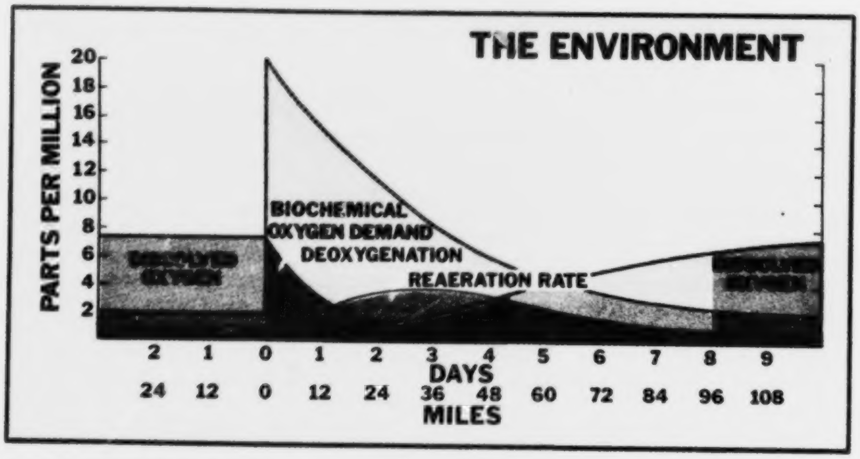


Figure 2 - The dissolved oxygen concentration in the stream is partially destroyed by the pollution load. Full depletion is avoided by reaeration processes.

rises again toward a restoration similar to that of upstream, unpolluted water.

The biochemical oxygen demand (BOD) curve is low in upstream, unpolluted water, increases at point O from the great charge of sewage and gradually decreases from this point downstream to a condition suggestive of unpolluted water. BOD and D.O. are so interrelated that the dissolved oxygen concentration is low where BOD is high, and the converse also is true. From left to right the stream zones are: clean water, degradation, active decomposition, recovery, and clean water.

EFFECTS OF REAERATION

Figure 2 represents an interpretation of the two principal antagonistic factors that have to do with the shape of the D.O. sag curve. The biochemical and other forces that tend to exhaust D.O. supplies, called collectively the process of deoxygenation, would reduce such resources to zero in about a day and one-half if there were no factors in operation that could restore oxygen to water. The river reach where D.O. would be completely gone would occur about 18 miles downstream from the point of discharge of sewage from the municipality. However, with reaeration fac-

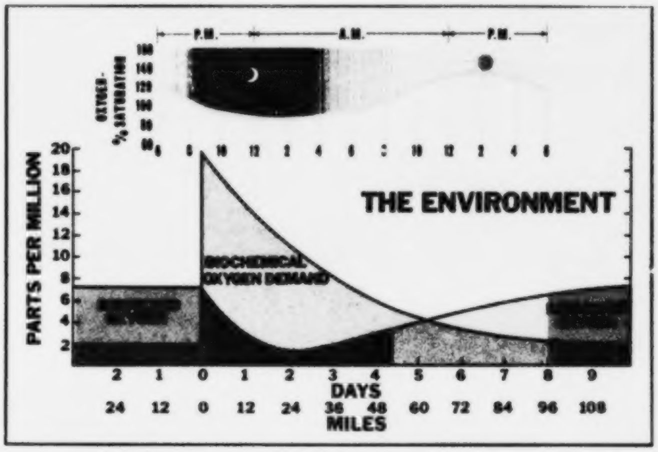


Figure 3 - Dissolved oxygen fluctuates according to available light, a result of photosynthesis. Thus, values on the lower curve are subject to daily variation.

tors at work, there is appreciable compensation for deoxygenation, and in this way the actual contour of the oxygen sag curve is determined. Thus, the low point of the curve is not attained at one and one-half days of flow at mile 18 with a zero D.O., but in reality is reached at about two and one-quarter days of flow at about mile 27. The D.O. here does not go to zero, but to 1.5 ppm.

If the population of the city remains fairly uniform throughout the year, and the flow is relatively constant, the low point of the D.O. sag curve can be expected to move up or down the stream with fluctuations in temperature. In winter, one can expect to find the low point farther downstream than shown. In other seasons, if temperatures exceed the 25°C upon which the charts are based, D.O. will be depleted more rapidly and drastically with the low point farther upstream.

The reach of any stream where the D.O. sag curve attains its low point obviously is the stream environment poorest in D.O. resources. It represents a place where aquatic life that may need a high D.O. can suffocate or from which such life may move to other stream areas where the D.O. resources are greater.

EFFECT OF LIGHT

The upper graph of Figure 3 illustrates fluctuations of dissolved oxygen that may occur over a 24-hour period at a single point in a stream with average density of aquatic greenery such as planktonic algae or larger submerged plants. For sake of explanation, any point in the recovery zone would exhibit such diurnal D.O. variations. The lower graph shows only linear changes in D.O., and gives no indication of the daily variation in availability of this vital gas that may occur at any single selected point.

If this selected point is in the recovery zone at mile 72, one can see from Figure 3 that D.O. varies from a low of about 80 percent saturation at 2:00 a.m. to about 140 percent at 2:00 p.m. Diurnal variation such as this is a result of photosynthesis chiefly in algae but in other plants also. During daylight hours these plants give off oxygen into the water in such large quantities that if the organic wastes are not sufficient to use up much of the D.O. in oxidizing sewage, the water commonly becomes supersaturated at some time during daylight hours. In addition to giving off oxygen, the photosynthetic process results in the manufacture of sugar to serve as the base from which flows the nutritional support for all stream life. The process of photosynthesis can be illustrated schematically as:

$$6\,CO_2 \quad 6\,H_2O \qquad C_6H_{12}O_6 \quad 6\,O_2$$

This action proceeds through the interaction of the green pigment, chlorophyll, contained in living plant matter, of sunlight, carbon dioxide, and even water to form the raw materials into a simple sugar and surplus oxygen.

While photosynthesis occurs, so also does respiration which proceeds 24 hours on end irrespective of illumination. In this well known process O_2 is taken in and CO_2 is given off. The algae, during daylight may yield an excess of oxygen over and above their respiratory needs, the needs of other aquatic life, and the needs for the satisfaction of any biochemical oxygen demand. Under these conditions, surplus oxygen may be lost to the atmosphere. During hours of darkness photosynthesis does not occur and gradually, the surplus D.O. that was present is used up or reduced by algae, fish, various insects, clams, snails and other aquatic life in respiration, and by bacteria in satisfaction of the BOD. That is why oxygen resources are poorest during early morning hours. During hours of darkness, a stream is typically dependent on physical reaeration for its oxygen resources after exhaustion of the "bank of dissolved oxygen," that was elevated to supersaturation levels by aquatic plants.

Obviously, on stream sanitary surveys where or-

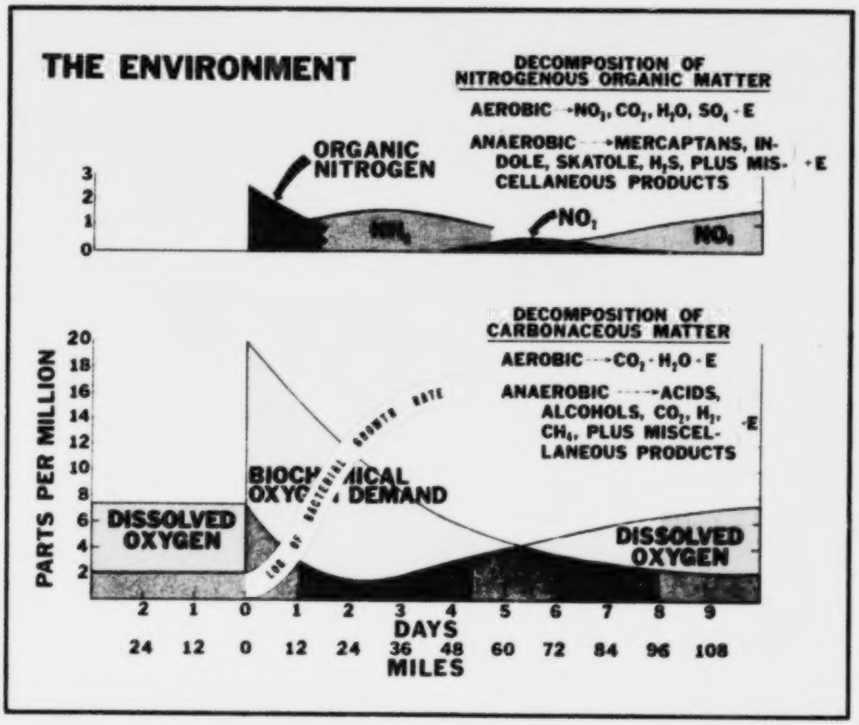


Figure 4 - With a heavy influx of nitrogen and carbon compounds from sewage, the bacterial growth rate is accelerated and dissolved oxygen is utilized for oxidation of these compounds. As this proceeds, food is "used up" and the BOD declines.

ganic wastes such as domestic sewage are pollutants, it is important to sample each station over 24 hours at intervals that are appropriate to reveal information on diurnal D.O. variations. If this is not done and station 1 is sampled consistently around 8:00 a.m. and station 6 around 5:00 p.m. over a weekly or a monthly survey, critical D.O. concentrations will not be found. If interval sampling over 24 hours cannot be done because of workday restrictions, reversing the time of sampling from the upstream to the downstream station on alternate days will at least show variations of D.O. that one can expect through an 8-hour workday.

EFFECT OF ORGANIC MATTER

The bottom graph of Figure 4 illustrates reasons for the decrease in the BOD curve progressively downstream and offers an explanation for the depression in the oxygen sag curve. On this graph there has been superimposed, in white, the shape of the log curve of bacterial growth rate. Accelerated bacterial growth rate is a response to rich food supplies in the domestic raw sewage. During rapid utilization of food, bacteria reproduction is at an optimum, and utilization of D.O. becomes fairly proportional to the rate of oxidation.

The upper graph illustrates, in principle, the progressive downstream changes in nitrogen from the organic form to the nitrate form. It demonstrates the initial high consumption of oxygen by bacteria that are feeding on proteinaceous compounds available in upstream waters in freshly discharged domestic sewage. With fewer and fewer of these compounds left in downstream waters, the BOD becomes reduced and the D.O. increases. Fat and carbohydrate foodstuffs rather than proteins could have been chosen just as well to show this phenomenon.

The nitrogen and phosphorus in sewage proteins can cause special problems in some receiving waters. Experience has shown that increasing the amount of these elements in water can create conditions especially favorable for growing green plants. In free flowing, clear, pebble brooks they appear as green velvety coatings on the stones or as lengthy streamers waving gently in the current. They are not unattractive and even, in the poetry of Nature, are complimented by the name "mermaid's tresses." These plants are not like the troublesome ones which occur mostly in more sluggish streams, impoundments or lakes, especially when they are artificially fertilized by sewage. In the clean brook, they not only are attractive and natural to see, but also they are a miniature jungle in which animals of many kinds prey upon each other with the survivors growing to become eventual fish food.

In more quiet waters, the algal nutrients in sewage are picked up for growth by less desirable kinds of algae. With great supplies of nitrogen and phosphorus made available, free-floating, minute blue-green algae increase explosively to make the water pea soup green, smelly and unattractive. In some unfortunate local-

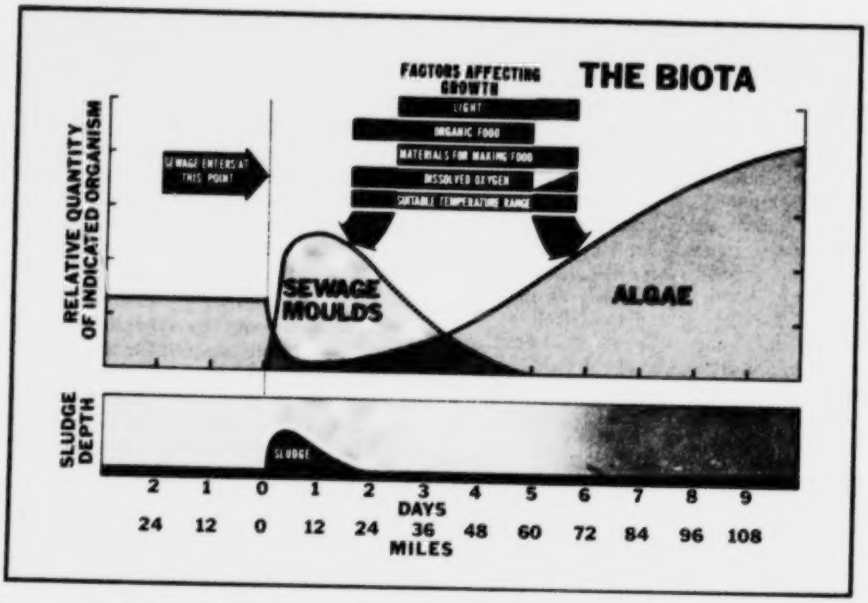


Figure 5 - Shortly after sewage discharge, the moulds attain maximum growth. These are associated with sludge deposition shown in the lower curve. The sludge is decomposed gradually; as conditions clear up, algae gain a foothold and multiply.

ities, nuisance blooms of algae have become so objectionable that waterfront dwellers have had to forsake their homes and see their property depreciate in value. The problem has been studied at a number of localities, and some studies are still in progress. Special legislation has even been formulated requiring that sewage treatment plant effluents not be discharged to susceptible lakes solely because of the algal nutrients they contain. Sometimes, under conditions not well understood, some blue-green algae develop poisons capable of killing livestock, wildlife and fish. Fortunately, such occurrences are rare. It is completely clear that sewage disposal and biological responses of even such lowly plants as algae go hand-in-hand sometimes to plague the desires of man.

AQUATIC PLANTS

In the lower part of Figure 5 a profile is shown of the water and stream bed with the vertical scale of the latter exaggerated. Sludge deposits begin to accumulate just below the point of sewage discharge. These deposits reach their maximum thickness near the point of origin but blanket the stream bed for many miles downstream. The substance of the deposits gradually is reduced by decomposition through the action of bacteria, moulds and other sludge-dwelling organisms, until it becomes insignificant about thirty miles below the municipality.

Also, at the outfall the water is turbid from fine solids held in suspension in the flowing water. Larger floating solids, destined to sink eventually to the stream bed as settleable solids, are visible on the water surface as they drift downstream. Both the fine and large solids contribute to the sludge deposit, and as they settle progressively to the bottom of the stream bed, the water becomes clear and approaches the color and transparency of upstream water above the point of sewage discharge.

The upper graph illustrates the relative distribution and quantities of algae, various moulds, and filamentous bacteria such as Sphaerotilus. From mile 0 to mile 36, high turbidity from floating debris and suspended solids is not conducive to algal production. Thus, except for slimy blue-green marginal and bottom types, algae are sparse in this reach. In order to grow well algae need sunlight, and here it cannot penetrate the water effectively. Also, floating solids that settle out of the water carry to the bottom with them floating algae that drift into the polluted zone from clear water areas upstream.

Blue-green algae that may cover marginal rocks in slippery layers and give off foul odors upon seasonal decay masquerade under the names: Phormidium, Lyngbya, and Oscillatoria. Green algae that accomodate themselves to the putrid zone of active decomposition frequently include Spirogyra and Stigeoclonium. Gomphonema and Nitzschia are among the diatoms that are present here.

Algae begin to increase in numbers at about mile 36. Plankton, or free-floating forms, steadily become more abundant and reach their greatest numbers in algal blooms some 40 to 60 miles farther downstream. This is where reduced turbidity, a lack of settleable sewage solids, final mineralization of proteinaceous organics to nitrate-nitrogen fertilizers, and favorable oxygen relations result in an ideal environment for growth of abundant aquatic plants.

Algae that may be found abundantly here may be represented by the bluegreen genera Microcystis and Anabaena; the pigmented flagellates are represented by Euglena and Pandorina; the green algae by Cladophora, Ankistrodesmus, and Rhizoclonium; and diatoms by Meridion and Cyclotella. Rooted, flowering, aquatic plants that form underwater jungles here are represented by the "water pest," Elodea, and various species of pond weeds known as Potomogeton. Such aquatic forests and meadows present an excellent natural food supply for the aquatic animals, and also serve them with shelter. Thus, commonly as plants respond downstream in developing a diversified population in the recovery and clean water zones, animals follow a parallel development with a great variety of species. In such reaches where the stream consists of numerous alternating riffles and pools, a great variety of fish are likely to occur.

In the reach where algae are scare (sic: scarce), from about 0 to mile 36, various moulds and bacteria are the dominant aquatic plants. Sphaerotilus filaments may abound in riffle areas at about mile 36 where physical attachment surfaces are available and where oxygen, although low, is adequate. Bacterial slimes may cover rocks and other submerged objects and bank margins. Such slimes have an abundant supply of available food in readily usable form of carbohydrates, proteins and fats and their digestion products. They are not bothered especially by high turbidities or by settleable solids. They do well living in the center of sludge or near it, in what to them is an "apple-pie" environment.

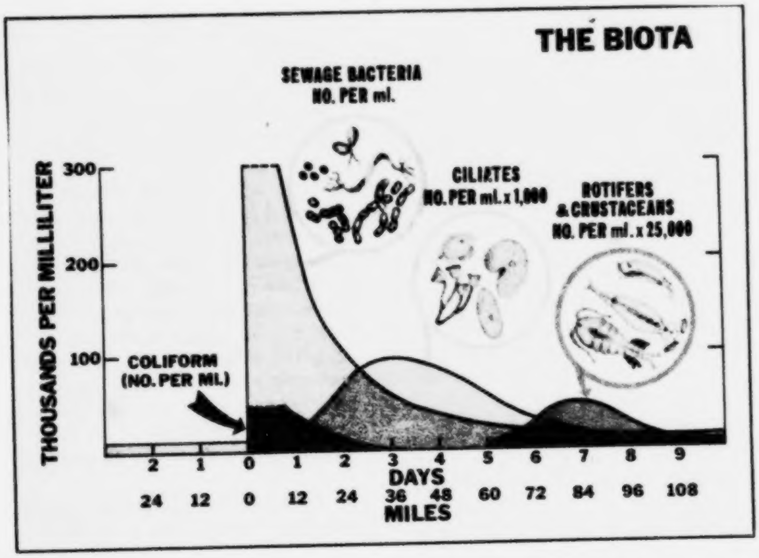


Figure 6 - Bacteria thrive and finally become prey of the ciliates, which in turn are food for the rotifers and crustaceans.

BACTERIA AND THE CILIATES

Associated with the bacterial slimes are certain ciliated protozoans that feed on bacteria and engulf small particles of settleable organic matter. Such ciliates are also found in aeration tanks of sewage treatment installations as a component of activated sludge and on the surface of rock in trickling filter beds. Common ones are Epistilis, Vorticella, Colpidium, and Stentor.

Figure 6 illustrates the interrelations between bacteria and animal plankton, such as ciliated protozoans, rotifers and crustaceans. The quantities shown and the die-off curves for sewage bacteria in toto and for coliform bacteria separately are theoretically accurate. The center curve for ciliated protozoans and the last curve representing rotifers and crustaceans are more accurate in principle than in actual quantities.

After entering the stream as a part of the sewage, bacteria, including coliforms, reproduce to become abundant in an ideal environment. Here they feed on the rich organic matter of sewage and by multiplying rapidly offer a ready food supply for ciliated protozoans which are initially few in number. After about a day of flow the bacteria may be reduced through natural die-off and from the predatory feeding by protozoans. After about two days of flow, the stream environment becomes more ideal for the ciliates, and they form the dominant group of animal plankton. After seven days, the ciliates fall victim to rotifers and crustaceans which represent the principal microscopic animal life in the stream.

It has been long suspected that the efficiency of this sewage consuming biological machine depends upon a close-knit savage society in which one kind of organism captures and eats another. Classical research of some time past showed that a single kind of bacterium mixed with sewage in a bottle could not do an efficient or rapid job of breaking down the sewage. Several kinds could do a better job, supposedly because one bacterial type, in acting upon parts of the sewage as food, prepared it for acceptance by another. With several bacteria a multilateral attack was made possible. But even a system like this is inefficient. Bacteria work best only when they are growing rapidly and they do this when they multiply frequently by splitting into two. It is important then that they not be permitted to attain a stable high and lazy population. In the bottle the task of stabilizing sewage goes most rapidly when ferocious bacteria-eating ciliates are introduced to keep the population at a low and rapidly growing state.

These relations between the bacteria eaters and their prey, discovered in the bottle, apply as well to efficient functioning of a modern sewage treatment plant. In some sewage treatment plants, examination is made routinely to see how the battle lines are drawn up between the bacteria eaters and their prey. It now becomes more obvious why sewage disappears so efficiently from the stream. It also is clear why the bacteria, the ciliates, the rotifers and the crustaceans increase, persist for awhile, and then decrease along the course of passage of the stream.

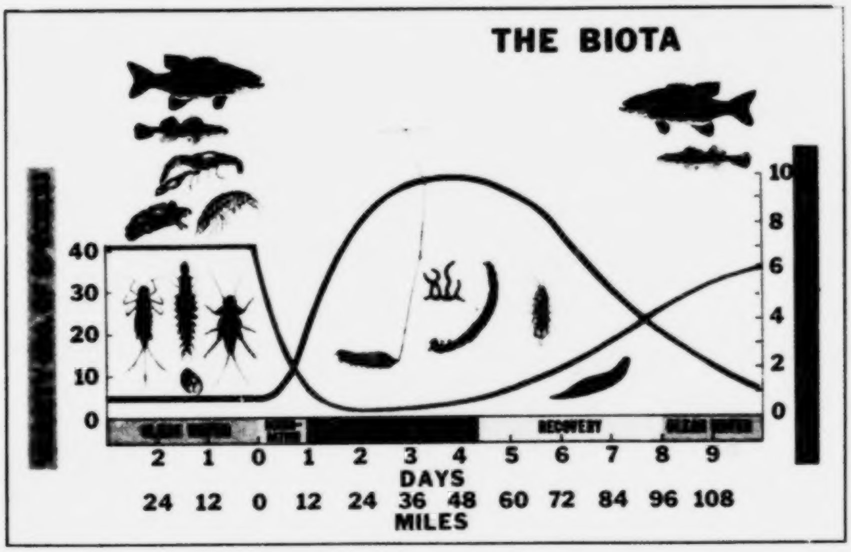


Figure 7 - The [upper] curve shows the fluctuations in numbers of species: the [lower] the variations in numbers of each.

THE HIGHER FORMS

Figure 7 illustrates the types of organisms and the numbers of each type likely to occur along the course of the stream under the assumed physical conditions that were stated earlier. The upper curve represents the numbers of kinds or species of organisms that are found under varying degrees of pollution. The lower curve represents the numbers of individuals of each species. In clean water above the city a great variety of organisms is found with very few of each kind represented. At the point of waste entry the number of different species is greatly reduced, and they are replaced by a different association of aquatic life. This new association demonstrates a severe change in environment that is drastically illustrated by a change in the species make-up of the biota. However, this changed biota, represented by a few species, is accompanied by a tremendous increase in the numbers of individuals of each kind as compared with the density of population upstream.

In clean water upstream there is an association of sports fish, various minnows, caddis worms, mayflies, stoneflies, hellgrammites, and gill-breathing snails, each kind represented by a few individuals. In badly polluted zones the upstream association disappears completely or is reduced, and is replaced by a dominant animal association of rattailed maggots, sludge worms, bloodworms and a few others, represented by great numbers of individuals. When downstream conditions again resemble those of the upstream clean water zone, the clean water animal association tends to reappear and the pollution tolerant group of animals becomes suppressed. Thus, clean water associations of animals may form parameters around polluted water reaches. Such associations may be indicative that water is fit for multiple uses, while the presence of a pollution tolerant association of animals indicates that water has restricted uses.

Pollution tolerant animals are especially well adapted to life in thick sludge deposits and to conditions of low dissolved oxygen. The rattailed maggot, Eristalis tenax, is not dependent on oxygen in water. This animal shoves its "snorkle-like" telescopic air tube through the water surface film to breathe atmospheric oxygen. Thus, even in the absence of oxygen it is one of the few survivors where most animals have suffocated. Those who have worked around sewage treatment installations have probably observed the flesh or milkish colored rattailed maggot in the supernatant over sludge beds where dewatering performance was poor. Commonly associated with it in this supernatant over sludge beds are the immature stages of the well-known "sewagefly," Psychoda, and wrigglers of the sewage mosquito, Culex pipiens. The rattailed maggot turns into a black and brownish banded fly about three-quarters of an inch long, called a "bee fly" because it closely resembles a bee. It differs by having two wings instead of four and does not sting. Sludge worms, Tubifex, are dependent upon the dissolved oxygen in water; however, they are well adjusted to oxygen famine and commonly are found in water with as little as half a part per million. They are actually aquatic earthworms, cousins of the terrestrial earthworms found in lawns and used as fish bait. These worms feed on sludge by taking it into the digestive tract. In passing it through their alimentary canal, they remove organic matter from it, thus reducing the biochemical oxygen demand. Sludge worms one and one-half inches long and as thick as a needle have been observed to pass fecal pellets totaling five feet nine inches through the digestive tract in 24 hours. Fecal pellets that are extruded from the anal openings have on occasion been found to have a biochemical oxygen demand of one-half of that of sludge that was not "worked-over" by them. The sludge worms are then, "actually crawling BOD," in that they incorporate sugars, proteins and fats that are present in sludge into their body cellular components. It may be difficult to visualize the magnitude of BOD removal that one worm, needle-thick in size and one and one-half inches long, can accomplish in relation to an extensive sludge deposit. However, when it is realized that from 7,000 to 14,000 of these worms may be found per square foot of bottom surface in sludges, considerable work is done in removing BOD. By the same token, for example, wrigglers of sewage mosquitoes, (Sic. .) Culex pipiens, that feed on the organics of sewage and emerge as adults to fly out of water represent BOD removed. In this instance it is "flying COD" that is factually taken out of water, whereas the crawling BOD of sludge worms is not removed, but is recycled back as the worms die.

The worm-like body of organisms composing the pollution tolerant association of the rattailed maggot, sludge worms, blood worms, and leeches is an ideal type to have for successful living in sludge. As settleable solids fall to the bottom, such organisms are not trapped and buried in them to die, but by wriggling with their worm-like cylindrical bodies, manage to maintain their position near the surface of sludge in communication with the water interface. Sow-bugs that are shown in Figure 7 with the "wormy-horde" do have well-developed appendages, but their life may be marginal on stream bank areas and on the surface of rocks protruding from sludge covered bottoms. Thus, they are not buried by settleable solids.

The invertebrates shown in clean water do not form successful populations in streams where settleable solids sink to form sludge deposits. Because their appendages may become clogged with sludge as solids settle, they may be carried readily to the bottom and be buried alive.

POPULATION FLUCTUATION

Figure 8 shows that the population curve of Figure 7 is actually composed of a series of population maxima for individual species. The species form a significant pattern in reference to each other and to the varying strength of the pollutant as it decreases progressively downstream. Sludge worms such as Tubifex and Limnodrilus can better withstand pollution than other bottom invertebrates. Thus, they reach great numbers closer to the source than other bottom dwelling animals. In turn they are replaced in dominance by red midges, also called bloodworms or Chironomids, and then by aquatic sow-bugs, Asellus. The sludge worms and red midges are so numerous in contrast to the other organisms shown in Figure 8 that

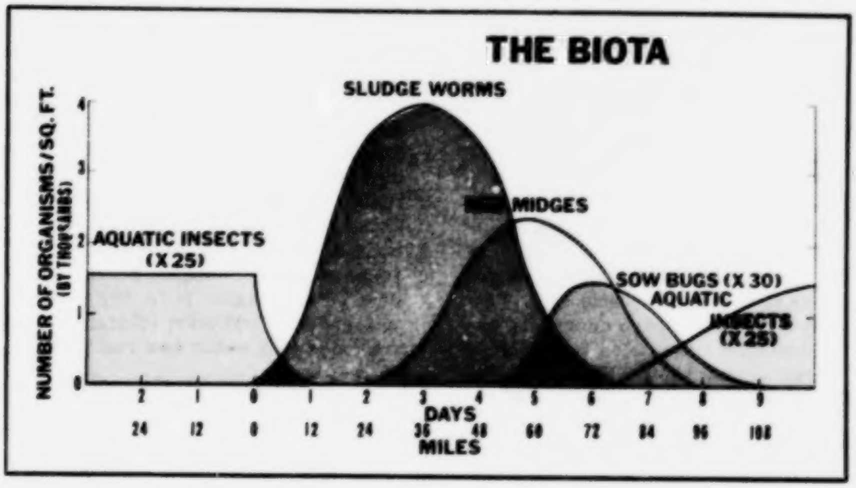


Figure 8 - The population curve of Figure 7 is composed of a series of maxima for individual species, each multiplying and dying off as stream conditions vary.

numbers of the latter are exaggerated 25 to 30 times to permit showing them effectively. Finally, when the effects of pollution have largely subsided in the environment, a variety of insect species represented by few individuals of each dominates the bottom habitat.

The story of pollution told here emphasizes that stream pollution and recovery may follow an orderly scheme under the influence of interacting physical, chemical and biological forces. Using streams as dumping places for sewage triggers the environmental and biotic changes that have been shown. These changes are not desirable. In most cases, in addition, they are hazardous to public health and otherwise impair the usefulness of valuable water resources. The needed remedy is to confine all of these interacting forces in an acceptable sewage treatment works so that this example of the Nation's water resources is protected for present and future use.

BIBLIOGRAPHY

1. Bartsch, A. F. 1948. "Biological Aspects of Stream Pollution." Sewage Works Journal, vol. 20, No. 2, pp. 292-302.
2. Brinley, Floyd J. 1942. "Biological Studies, Ohio River Pollution, I. Biological Zones in a Polluted Stream." Sewage Works Journal, vol. 14, No. 1, pp. 147-152.
3. Brinley, Floyd J. 1943. "Sewage, Algae and Fish." Sewage Works Journal, vol. 15, No. 1, pp. 78-83.
4. Claassen, P. W. 1932. "The Biology of Stream Pollution." Sewage Works Journal, vol. 4, No. 1, pp. 165-172.
5. Eliassen, R. 1952. "Stream Pollution." Scientific American, vol. 18, No. 3, pp. 17-21.
6. Hubbs, C. L. 1933. "Sewage Treatment and Fish Life." Sewage Works Journal, vol. 5, No. 6, pp. 1033-1040.
7. Ingram, W. M. 1957. Handbook of Biological References on Water Pollution Control, Sewage Treatment, Water Treatment. Public Health Service Publication No. 214 (Revised 1957), pp. 1-95.
8. Katz, M. and A. R. Gaufin. 1953. "The Effects of Sewage Pollution on the Fish Population of a Midwestern Stream." Transactions American Fisheries Society, vol. 82, pp. 156-165.
9. Lackey, J. B. and C. N. Sawyer. 1945. "Plankton Productivity of Certain Southeastern Wisconsin Lakes as Related to Fertilization. I. Surveys." Sewage Works Journal, vol. 17, No. 3, pp. 573-585.
10. Lackey, J. B. 1945. "Plankton Productivity of Certain Southeastern Wisconson Lakes as Related to Fertilization. II. Productivity." Sewage Works Journal, vol. 17, No. 4, pp. 795-802.
11. Olson, T. A. 1932. "Some Observations on the Interrelationships of Sunlight, Aquatic Plant Life and Fishes." Read at Sixty-second Annual Meeting, American Fisheries Society, Baltimore, Maryland, pp. 1-11.
12. Purdy, W. C. 1926. "The Biology of Polluted Water." Jour. Amer. Water Works Assoc., vol. 16, No. 1, pp. 45-54.
13. Richardson, R. E. 1928. "The Bottom Fauna of the Middle Illinois River, 1913-1925." Bull., Illinois Natural History Survey, vol. 17, No. 2, pp. 387-475.
14. Streeter, H. W. and E. B. Phelps. 1925. "A Study of the Pollution and Natural Purification of the Ohio River. III. Factors Concerned in the Phenomena of Oxidation and Reaeration." Public Health Service Bulletin No. 146, pp. 1-75.
15. Suter, R., and E. Moore. 1922. "Stream Pollution Studies." State of New York Conservation Commission, Albany N. Y., pp. 3-27.
16. Tarzwell, C. M. and A. R. Gaufin. 1953. "Some Important Biological Effects of Pollution Often Disregarded in Stream Surveys." Purdue University Engineering Bulletin, Proc. 8th Industrial Waste Conference (May 4-6, 1953), pp. 295-316.

Chapter IV

RELATIONSHIP TO POLLUTION OF FISH

Reproduced With Permission From:
BULLETIN OF THE BUREAU OF FISHERIES
48(1937) : 365-437

DETECTION AND MEASUREMENT OF STREAM POLLUTION*

M. M. Ellis, Ph.D., Sc.D.
In charge, Interior Fisheries Investigations, United States Bureau of Fisheries,
and Professor of Physiology, University of Missouri

CONTENTS

Page
- Introduction ... 129
- Stream pollutants and aquatic environment ... 130
  - Physical and chemical characteristics of waters suitable for fresh-water stream fishes ... 130
    - General field methods ... 131
      - Water samples ... 131
      - Mud samples ... 131
      - Plankton ... 131
      - Fresh-water mussels ... 131
      - Fish ... 134
    - Equipment ... 134
    - Dissolved oxygen ... 134
    - Hydrogen-ion (pH) limits ... 141
    - Ionizable salts ... 144
      - Specific conductance ... 144
      - Carbon dioxide ... 145
        - Fixed carbon dioxide (chiefly calcium and magnesium carbonates) ... 145
        - Free carbon dioxide ... 148
      - Iron ... 149
      - Ammonia ... 150
      - Suspensoids ... 152
      - Depths ... 153
      - Temperature ... 153

Page
- Stream pollutants, etc. - Continued
  - Bottom conditions as affected by stream pollution ... 154
- Action of pollutants on fishes ... 156
  - Injuries to gills and external structures ... 156
  - Pollutants entering the body of the fish and exerting true toxic action ... 157
- Lethality of specific substances occurring in stream pollutants ... 160
  - General consideration ... 160
  - Test animals ... 160
    - Goldfish ... 161
    - Daphnia magna ... 161
  - Water types ... 163
  - Specific lethality tables ... 164
    - Osmotic pressure and sodium chloride ... 164
    - Acids ... 164
    - Compounds of various metals ... 167
    - Miscellaneous compounds ... 170
- Lethal limits of 114 substances which may be found in stream pollutants ... 171
- Acknowledgments ... 182
- Bibliography ... 182

INTRODUCTION

The menace of pollution to our inland streams and rivers is too well known to require definition. In fact, unsightly and noisome conditions due to pollution are encountered so often that they have come to be accepted by many as the usual order of things. It is true, however, that many cases of pollution could be remedied and the streams so affected restored to an acceptable state for recreation, fishing, and general use with reasonable expense, if all parties concerned would cooperate. To obtain this cooperation, it is necessary to understand the situation and to judge it fairly. Much confusion and misunderstanding has arisen in attempts to define the extent of pollution and to place the responsibility for damage to fisheries, because of the lack of available information on the conditions to be defined.

In the present paper findings from the widely scattered scientific literature supplemented by the experimental and field work of various agencies have been brought together covering (1) the conditions which should be maintained if good fish faunae are to thrive, and (2) the specific effects of various types and components of effluents which now pollute our streams.

It is hoped that the use of this information will make possible the definition of undesirable conditions with fairness both to the industrialist, who must use water and streams, and to the citizen, who is entitled to enjoy these same streams.

With the limits of both the required stream conditions and of the pollutant lethalities better understood, corrective measures can be recommended intelligently, for remedial action can only be instituted when the cause and the severity of the pollution are known. The United States Bureau of Fisheries is engaged at present in such investigations based on findings presented here.

---

*Bulletin No. 22. Approved for publication, Sept. 1, 1936. The present study is presented as the first of three dealing with pollution hazards to fresh-water fishes. The second will discuss trade wastes and chemical effluents, and the third, the cumulative effects of dilute pollutants.

STREAM POLLUTANTS AND AQUATIC ENVIRONMENT

Physical and Chemical Characteristics of Waters Suitable for Fresh-Water Stream Fishes

The various effluents, municipal, industrial, and otherwise which comprise collectively stream pollutants may be detrimental to fishes and other aquatic life either indirectly through quantitative alterations in those substances which give fresh waters their inherent characteristics, as dissolved oxygen, carbonates, and hydrogen ions, or directly because of specific physiological and toxic effects on the aquatic organisms themselves. Many effluents are of complex composition, however, and are harmful to aquatic life through both changes in the aquatic environment and through definite toxic actions. Therefore, in determining the effects of stream pollutants on aquatic biota and particularly on fishes, it has been necessary to study both the modifications in the environment and the specific physiological actions attributable to the different pollutants.

The many substances which are carried in solution and suspension by a stream, collectively determine whether the waters of that stream in themselves present conditions favorable or unfavorable for fishes and other aquatic organisms; and any individual fish in the stream is affected not only directly by these substances, but indirectly through their action on other forms of aquatic life which comprise in a very restricted environment the food, the enemies, and the competitors of the particular individual. The definition of the amounts of these substances which should be present in water in order to maintain a suitable environment for fishes, or which may be tolerated by fishes under favorable conditions, is therefore much more involved than the designation of standards for water for human consumption, which concern but a single, air-breathing, non-aquatic animal, man.

Water standards for fishes and other aquatic organisms, moreover, are not identical with those standards which will define water as potable for human beings or satisfactory for industrial use. Water may be serviceable for many industries and yet not support fish life, or fishes may thrive in water which would be unsafe for human consumption due to the presence of particular bacteria as typhoid, or certain compounds harmful to man, as the western alkalis.

The definition of waters as suitable for aquatic life is complicated still further by the fact that various species of fishes and other aquatic animals and even individuals of different ages of the same species have different degrees of tolerance to deviations from the ideal environment, and to the cumulative effects of many stream pollutants. Consequently, the presence or even the survival for a time of fishes in waters suspected of pollution does not in itself constitute evidence that these waters are either satisfactory or safe for fishes.

In spite of the various confusing factors which have been set forth in the preceding paragraphs, it is essential in order to determine the extent and degree of pollution in any given stream to define as far as possible the limits of variation in the several components of those aquatic complexes which desirable fishes will tolerate and in which they will still thrive. Neither minimal lethal nor arbitrary standards will suffice. The limiting values for the various substances in stream waters, with references to the effects on aquatic life, as presented here have been obtained through the correlation of data of four sorts: (a) The amounts of these substances found in natural waters where fishes were successfully maintaining themselves, (b) studies of streams which as far as could be determined were unpolluted and which, therefore, presented natural conditions, (c) the physiological responses of fishes and other aquatic animals to variations in the concentrations of these substances, and (d) the survival of aquatic forms when exposed to these substances over long periods under controlled conditions. These data have been drawn from the existing literature, and from field and laboratory studies by the staff of the Columbia (Mo.) field unit of the United States Bureau of Fisheries during the past 5 years (Ellis, 1935a).

In the present consideration of water standards for fish and other aquatic animals the dissolved and suspended substances have been divided into two groups, namely, those constituting the complex favorable to fishes in natural unpolluted waters, i.e., those substances to which the fresh-water fishes are physiologically adapted; and, those substances which are added from time to time to natural waters by man and his agencies, and to which the individual fish must adapt itself. There is, of course, some overlapping between the two groups since certain forms of pollution merely alter the amounts of specific substances normally found in streams, as in the case of the acid wastes from wire-nail mills, which effluents raise the acid ions, the iron and the sulphates, all of which occur in small quantities in most streams, to levels toxic or detrimental for aquatic forms, with, of course, disastrous results.

Throughout the application of these data and standards it must be borne in mind that individual fishes and various species of fish have different degrees of resistance and tolerance. Consequently, some fishes may be found in waters where less favorable conditions than those here designated obtain, since both the minimal and maximal limits immediately compatible with life have been avoided, for these limits cannot be regarded as desirable or physiologically reasonable in determining a suitable environment for fishes any more than for man. An effort has been made, therefore, to present usable and reasonable standards of water suitability favorable to fish life; that is, standards defining waters in which a mixed fauna of fresh-water fishes of the common warm water types including desirable centrarchids, cyprinids, catostomids, and silurids, as well as such tolerant forms as carp and gar, will thrive. Various lethal limits are also set forth.

It must also be pointed out that these standard of suitability must be maintained throughout the periods of low water, maximal temperature, and maximal liability to pollution, since a deviation in the amount of any of several substances, as dissolved oxygen, acids, or salts, to the critical level for only a few hours may so change conditions in a considerable portion of an otherwise favorable stream, that months or years may be required to reestablish the former fish fauna and normal balance of aquatic species.

In a large series of field studies it has been shown that the natural, inherent water conditions of most streams can be ascertained satisfactorily for pollution studies as regards fisheries problems by determining repeatedly at different times of the night and day and at various seasons of the year the (1) dissolved oxygen, (2) pH, (3) ionizable salts, (4) carbon dioxide, fixed and free, (5) total ammonia, and (6) suspensoids, since the determinations of these factors not only give specific data concerning particular conditions, but also concerning several complexes which vary in even unpolluted streams and which are definitely affected by many forms of pollution.

From determinations made at many stations where good mixed fish faunae were present, it was found that the values from the above determinations in favorable waters, i.e., waters supporting good mixed fish faunae, fell within rather definite limits, and that deviations from these limits in our inland streams were almost always indicative of conditions unfavorable to aquatic life. However, these values alone, which cover only the more basic, inherent conditions which must be maintained in any stream if it is to support a good fish fauna and on which conditions of specific pollution are superimposed, will not suffice for the complete definition of water as favorable for aquatic life, since the absence of specifically toxic substances must also be demonstrated before the water can be finally approved as unpolluted.

GENERAL FIELD METHODS

Throughout these studies, both for the determination of water suitability standards and of pollution conditions, certain routine procedures were followed, in addition to the special investigations which the conditions in the particular locality required, in order that certain data from all localities could be compared fairly and without the skewing which results from haphazard sampling.

The stations at which samples were taken in each locality were selected as representative of the various complexes of conditions presented. Whenever possible, samples were taken at intervals throughout the night and day and at different times during the season. Many of the stations included in this report have been visited repeatedly during the past 5 years.

Although in this paper the detailed data concerning the findings on plankton, bottom organisms, fresh-water mussels, and fish population are not presented, since various general statements concerning findings on these animals have been included and since the studies of these forms constitute part of the routine background for the interpretation of the pollution hazards, the field methods employed in connection with the entire pollution study operations have been given.

WATER SAMPLES

At each station water samples were collected by means of a brass sampler of the general type described by Kemmerer, Bovard, and Boorman (1923) and transferred at once without aeration to 300-cubic-centimeter glass-stoppered, self-sealing magnesium-citrate bottles. The first or top water samples were taken at a depth of form 1 to 2 feet, the actual surface water (the first few inches below the surface) being avoided in the general sampling because the dissolved gases in the water in immediate contact with the air do not present a true picture of the dissolved gases in the main mass of the water in the stream (this fact is discussed more fully under dissolved oxygen). Whenever the depth was greater than 3 feet a second set of water samples, bottom samples, were taken about 3 inches above the floor of the stream. Intermediate samples were collected between the top and bottom at levels from 5 to 10 feet apart wherever the depth of the water justified such sampling.

MUD SAMPLES

Bottom mud samples were obtained with a Peterson dredge properly weighted to bring up about one-half cubic foot of bottom from an area approximately 1 foot square. These mud samples were divided into aliquot parts, some of which were dried (see Fig. 3) or fixed with various reagents for chemical determinations, and others were sieved through a series of Monell screens for qualitative and quantitative studies of the bottom fauna.

PLANKTON

Plankton counts were made from the catch obtained by pumping slowly 1 cubic meter of the water under consideration through a standard silk bolting cloth plankton net, supported in the water and provided with a glass trap bottle of 250-cubic-centimeters capacity in which the organisms accumulated uninjured. On some of the river lakes, as Lake Pepin and Lake Keokuk, a trawl supported by a boom and operated over the side of a cruiser was used both for plankton and the larger surface animals.

For purposes of quick field diagnosis of water conditions only net plankton were used, although in the detailed studies of plankton both nano- and macroplankton were determined.

FRESH-WATER MUSSELS

At the stations where fresh-water mussels were to be taken a heavy mussel dredge (see Fig. 4) operated from a catamaran was used. This dredge, which was found to be very effective to a depth of 8 or 9 inches in the mud of the river floor, brought up approximately 1-1/2 cubic feet of river bottom with the contained organisms. Quantitative as well as qualitative studies were possible, therefore, from these dredgings.

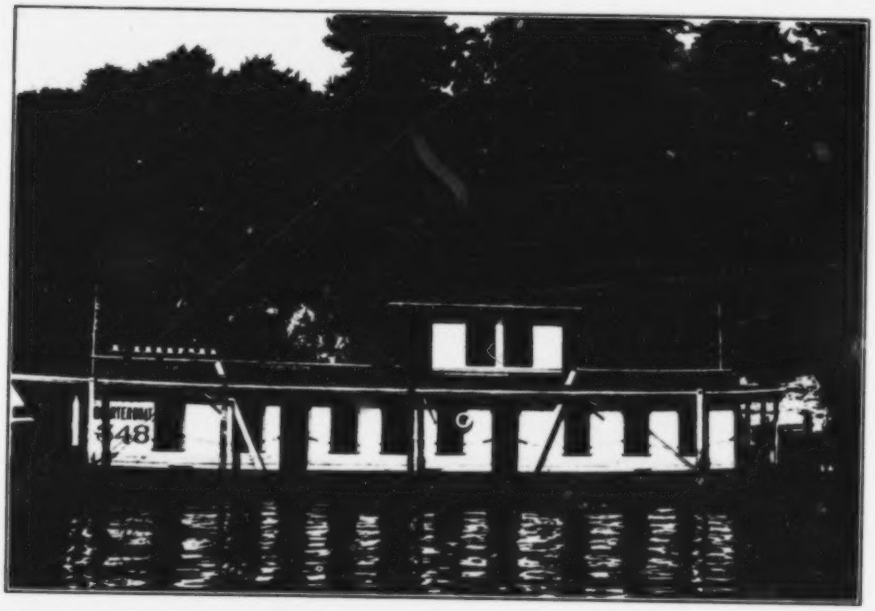


Figure 1 - U. S. Quarterboat 348, the floating laboratory of the United States Bureau of Fisheries.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

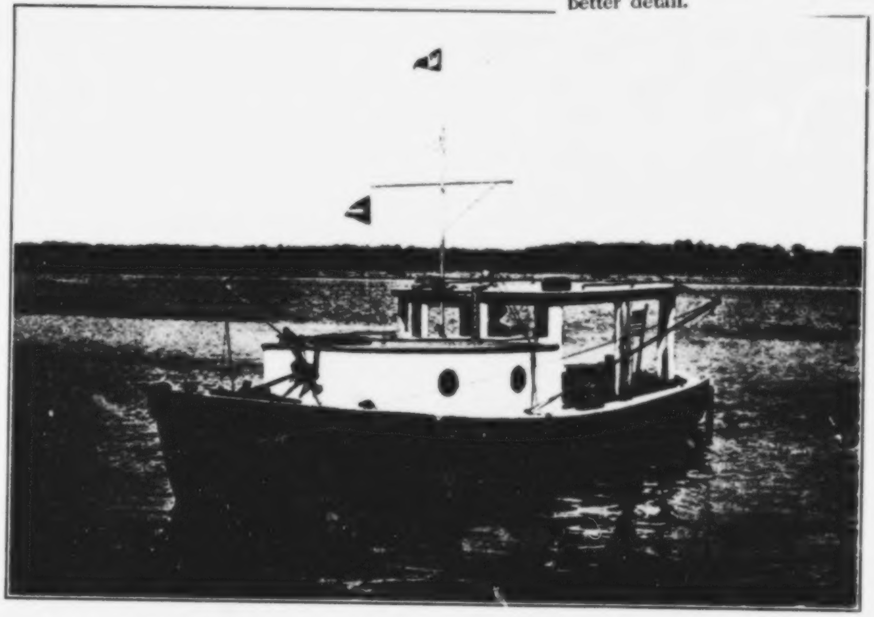
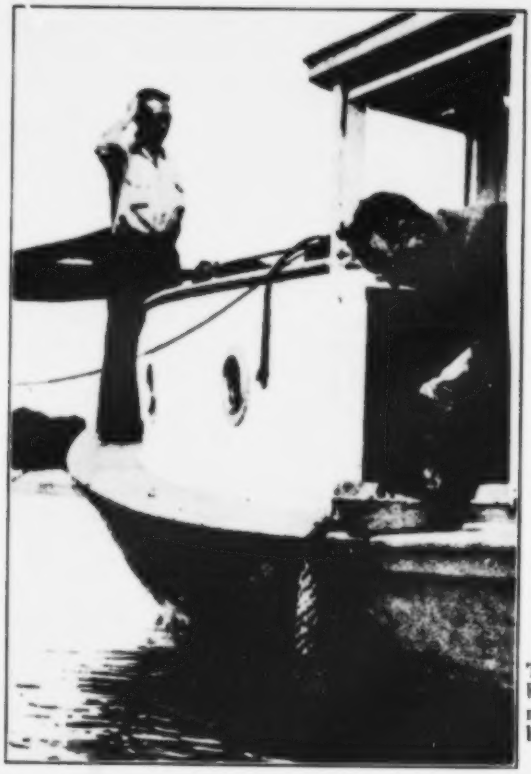


Figure 2 - U. S. Fisheries cruiser 56, equipped with sounding and sampling booms and field-laboratory apparatus. Two such cruisers were used on the larger rivers and streams.



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 3 - Collecting bottom samples from cruiser with Peterson dredge.

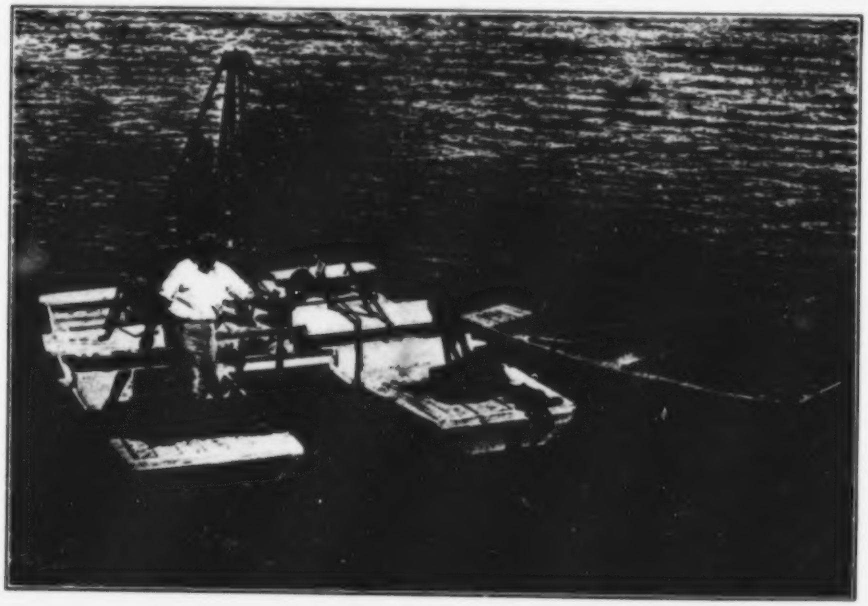


Figure 4 - Mussel dredge in operation from catamaran. This dredge was used in the study of bottom conditions where rocks and other obstructions made use of Peterson dredge impossible.

FISH

The fish fauna was studied from collections made with various types of nets and seines. Several hauls were made in each of the various portions of the habitat under consideration and usually the entire catch preserved. If the entire catch were too large, representative collections from it were made and the total bulk of the catch noted.

EQUIPMENT

In the field studies reported in this discussion several types of equipment have been used. In the major stream surveys U.S. Quarterboat 348 (see Fig. 1), which is fully equipped with chemical and hydrobiological laboratories, together with living quarters for 12 people, was used as the base from which the operations were conducted. Attached to the quarterboat for these investigations were two 35-foot cruisers (U.S. Fisheries 53 and U.S. Fisheries 56, see Fig. 2) which were specially equipped with dredging, sounding, and sampling apparatus, together with portable chemical units for such determinations and preparations as required immediate attention in the field. On certain streams where it was not feasible to move the quarterboat and cruisers, support and cooperation in equipment was given by various Government, State, and private agencies, including the loan of vessels and the use of equipment at hand.

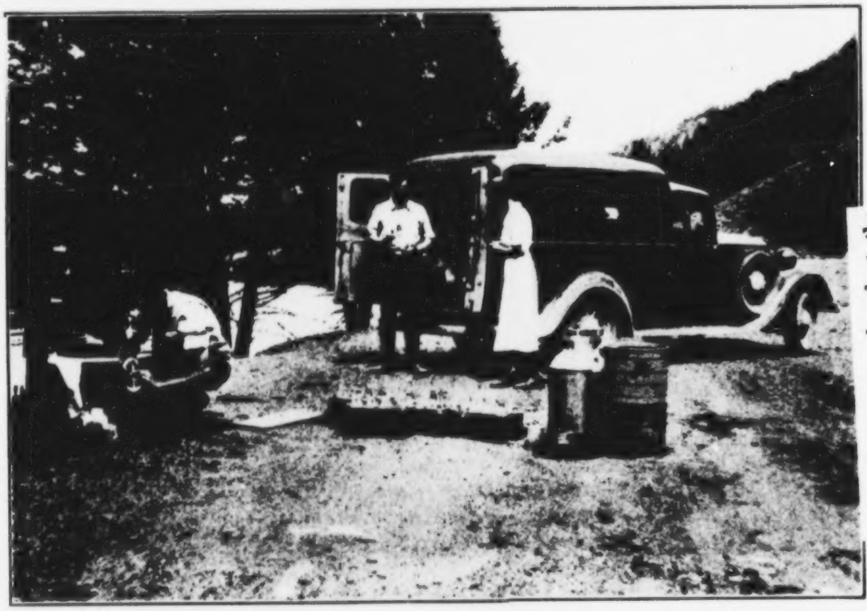
For certain stream studies automobile trucks carrying compact biochemical, biophysical, and hydrobiological apparatus were also used extensively. These mobile units were found to be very effective, as long distances could be covered quickly and the equipment taken to the specific site of the pollution problem to be investigated. (See Fig. 5.)

The detailed experiments, most of the bioassay work, and various analyses and tests which could not be made in the field were conducted at the United States Bureau of Fisheries laboratories at the University of Missouri. Here, through the excellent cooperation afforded by the University of Missouri, the Bureau has a research laboratory suite of eight rooms specially equipped for biochemical, physiological, and biological work, in which various pieces of apparatus devised particularly for these pollution studies are in operation.

DISSOLVED OXYGEN

The modifications of the Winkler method for oxygen determinations as described by Kemmerer, Bovard, and Boorman (1923) and as given by American Public Health Association (1933) were followed in both the field and laboratory analyses for dissolved oxygen. The data are reported in parts per million (p.p.m.) unless otherwise specified.

In Figures 6, 7, 8, and 9 are presented the results of 5,809 determinations of dissolved oxygen made at 982 stations on fresh-water streams and rivers of the United States during the months of June to September, inclusive, 1930-35. Natural lakes and ponds are excluded, the data covering only conditions in stream and river waters during the warm season. This season was chosen since the oxygen carrying power of water decreases and the metabolic demands of aquatic



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 5 - One of the field-laboratory trucks, showing stream-side operations. These trucks were equipped with apparatus and chemicals, sampling and collecting outfits, dredges, and seines. Each truck unit was able, therefore, to make detailed investigations at any stream site which could be reached by car.

animals for oxygen increase as the temperature of water rises.

From these 5,809 cases, all determinations made at stations where good, mixed, fish faunae were found at the time of sampling, were selected for a composite regardless of the actual amount of dissolved oxygen present. For purposes of this comparison good, mixed, fish faunae were defined as faunae including representatives of the fine fish group (trout, or bass, sunfish, perch, and other spiny-rayed fishes), of the rough fish group (suckers, buffalo, and catfish), and of the minnow series, in good condition at the time the samples were taken. Naturally, the exact species composition of these good, mixed faunae varied to some extent with the river system.

This composite selected as explained, includes 1,297 determinations of dissolved oxygen from 372 stations. Figure 6A gives the distribution of the dissolved oxygen values for this composite. To avoid skewing due to uneven number of cases from the different localities the various river systems were given equal weights in determining the percentages.

From Figure 6A it may be seen that during the warm season the waters at 96 percent of the good fish faunae stations carried 5 p.p.m. or more dissolved oxygen, and that in all of the 5,809 cases good, mixed fish faunae were not found in waters carrying less than 4 p.p.m. dissolved oxygen. These data collected from localities where the fish had had opportunity to choose for themselves point very strongly to 5 p.p.m.

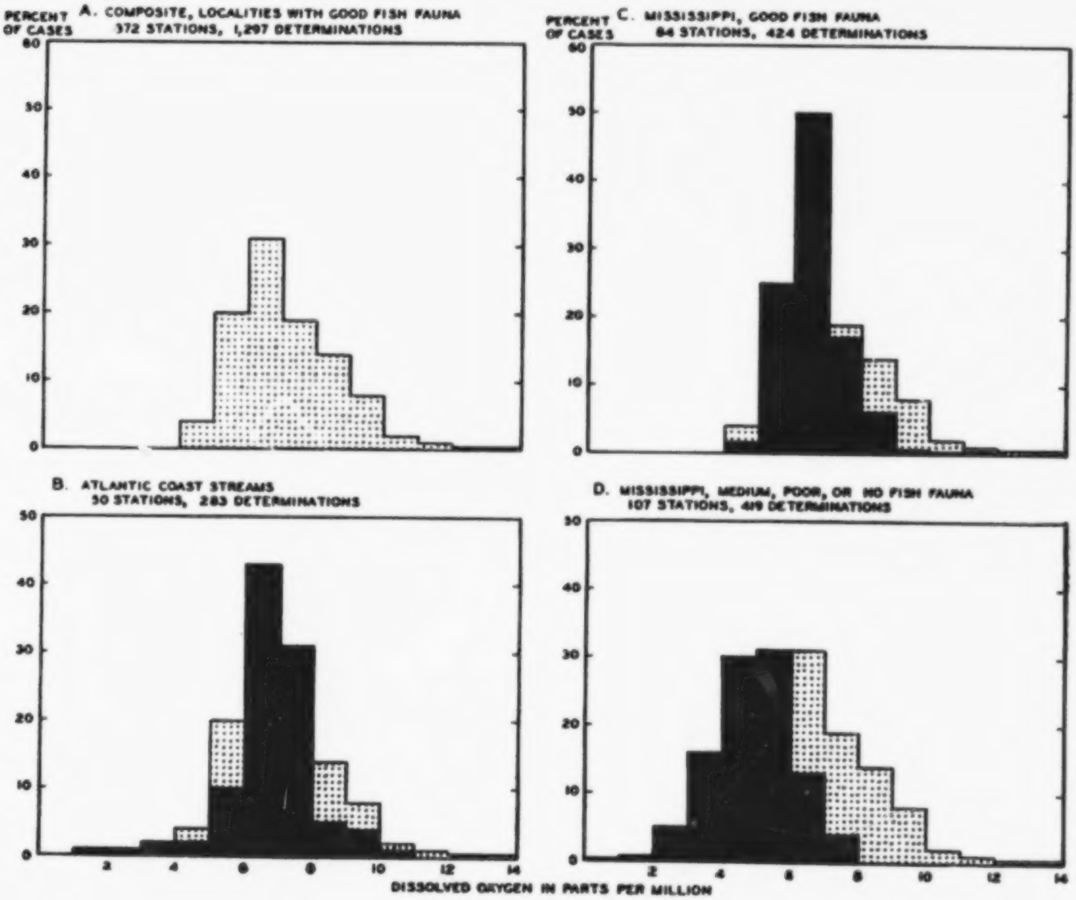


Figure 6 - The stippled graph is a composite of dissolved oxygen values for all stations at which good mixed fish faunae were found, each river system being given equal weight to avoid skewing due to differences in actual numbers of cases. Each solid black graph presents all oxygen data for a single river unit regardless of presence or absence of fish at sampling stations. Direct evaluation of each river unit can be made in terms of the preferences of good fish faunae from the stippled graph on which the black graph is superimposed.

as the lower limit of dissolved oxygen, if the complex is to maintain a desirable fish faunae under natural river conditions.

It may be suggested in the absence of other data that the aggregation of the good fish faunae in waters containing 5 p.p.m. or more dissolved oxygen does not constitute proof that this amount of oxygen was required by these fishes, and that some other factor or factors delimit these complexes where good fish faunae were found. However, experimental data presented in another portion of this section support the view that dissolved oxygen is definitely one of the determining factors in these favorable complexes.

A comparison of Graphs C and D in Figure 6, with the composite A, however, brings out the fallacy, for river waters at least, of the suggestion which has been made at times that the determination of dissolved oxygen or dissolved oxygen in terms of oxygen demand is sufficient alone to define water as suitable or unsuitable for fish life. Graph 6C gives the distribution of the dissolved oxygen value for all stations in the Mississippi River where good fish faunae were found and Graph 6D for all stations in the Mississippi River where medium, poor, or no fish faunae were found regardless of type of pollution. In this comparison the water at over 98 percent of the stations where good fish faunae were found carried 5 p.p.m. or more dissolved oxygen, and the water at 52 percent at stations where medium, poor, or no fish faunae were found carries less than 5 p.p.m. dissolved oxygen, but the water at 48 percent of the stations where medium, poor, or no fish faunae were found because of other unfavorable conditions, carried 5 p.p.m. or more dissolved oxygen due to current action and other factors in spite of the polluting materials.

Three other observations may be made from these studies of warm-season dissolved-oxygen values in the various streams and river lakes. Low dissolved

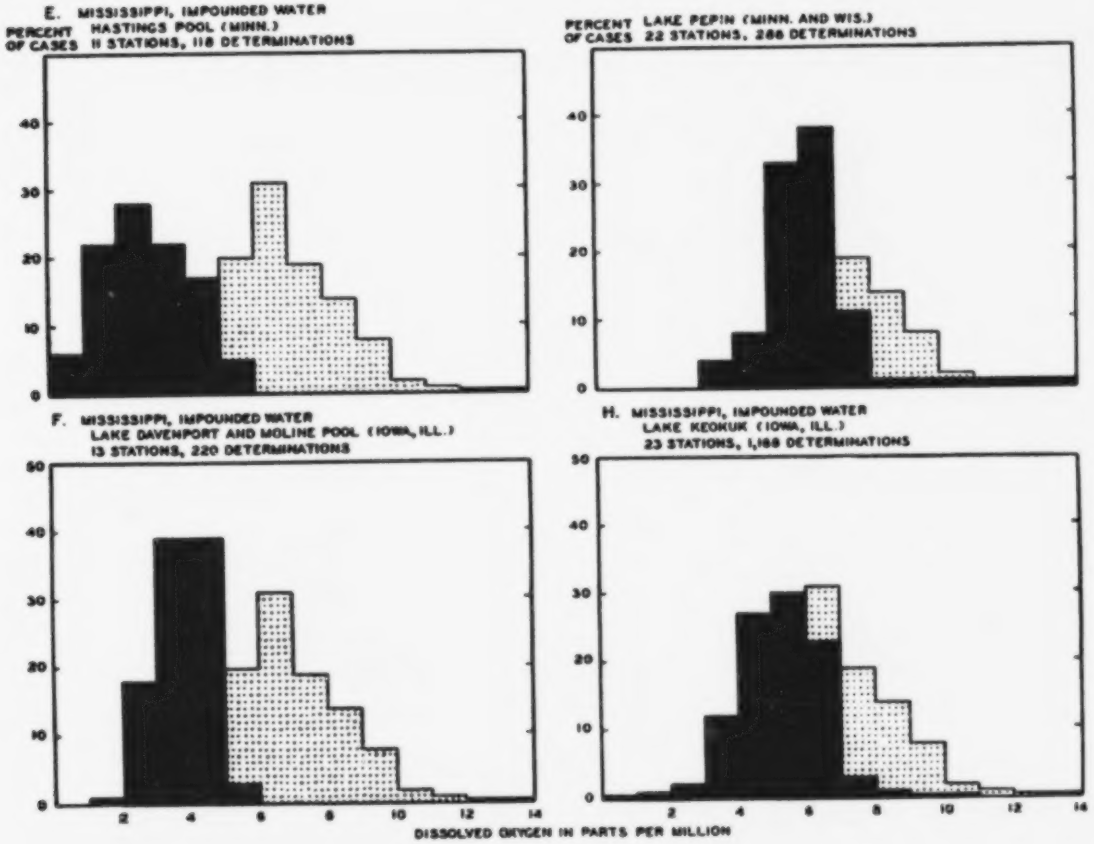


Figure 7 - Continuation of the dissolved oxygen comparisons, stippled Graph A in Figure 6, being the standard.

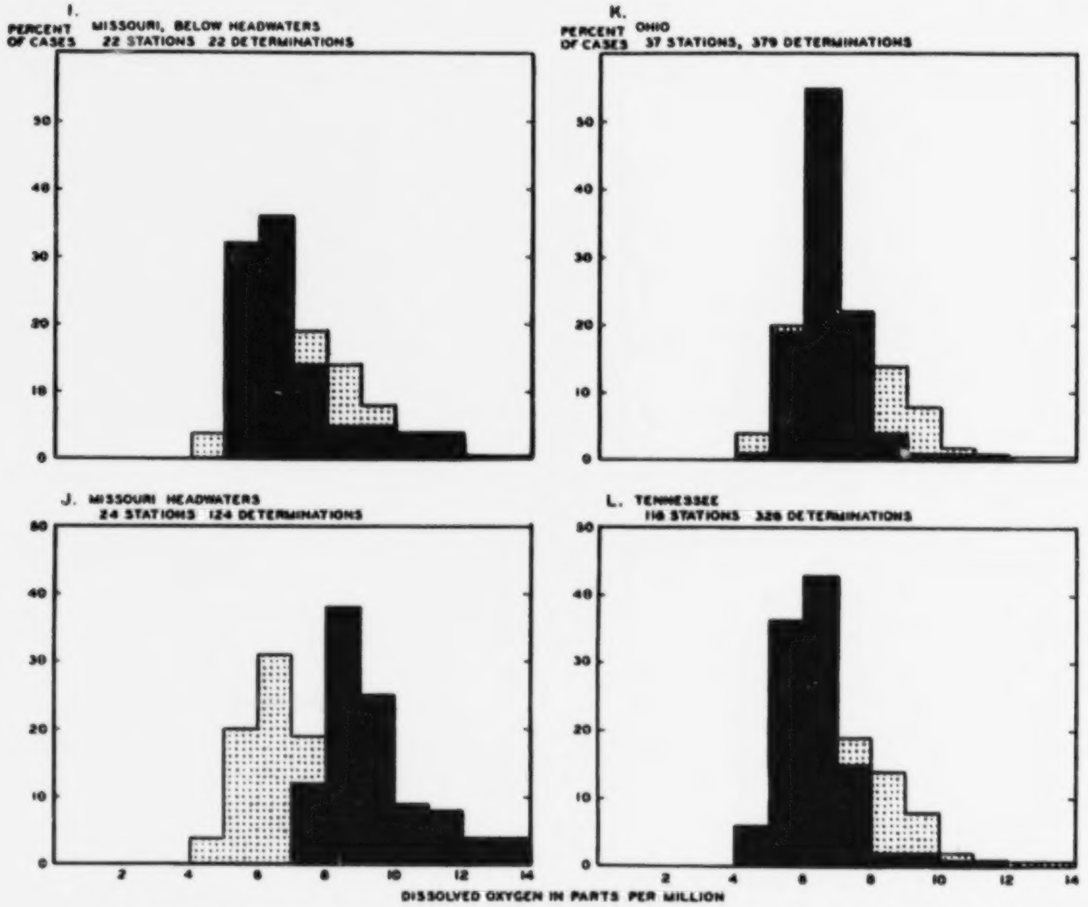


Figure 8 - Continuation of dissolved oxygen comparisons, stippled Graph A in Figure 6, being the standard.

oxygen was found more frequently in the impounded waters of streams, particularly if the impounded waters were subject to organic pollution, than in the more rapidly flowing portions of these same streams (see Graph 7E of Hastings Pool, a notoriously polluted portion of the Mississippi River, and Graphs 7F and 7H); headwater streams, especially those in mountainous regions usually have high dissolved oxygen, and the range of dissolved oxygen to be expected in flowing streams both polluted and unpolluted is in general from 0 to 14 p.p.m.

For the final evaluation of the various findings presented from the field studies on dissolved oxygen, experimental data covering the oxygen requirements of fresh-water fishes must also be considered, as both the amount of oxygen consumed by the fish and the minimal amount of dissolved oxygen which will barely support life vary with the combinations of environmental factors operating at the time, and with the size and species of the fish. Temperature, pH, and dissolved carbon dioxide are particularly important in this connection.

Many observers have pointed out that the metabolism of, and consequently the oxygen consumed by, fishes and other aquatic animals follows in general the van't Hoff law with reference to temperature so that the actual amount of oxygen removed from the water by the individual fish will vary with the temperature regardless of the amount of oxygen present until a near-lethal point is reached (Keyes, 1930). Ruttner (1926) states that the oxygen consumption of many aquatic animals is almost doubled with each rise of 10° C., within physiological limits, and Powers (1922 and 1932) has pointed out correlations between the utilization of oxygen by fishes at low oxygen tensions and the pH and carbonate systems.

The variation in the amount of oxygen consumed by fresh-water fishes of different species is apparent from the work of Gardiner, King, and Powers (1922) who give the oxygen consumption of the brown trout as from 90 to 200 cubic centimeters per kilo of body weight per hour at temperatures between 4° and 20° C.; of the goldfish as 16 to 90 cubic centimeters; and of the eel 9 to 60 cubic centimeters.

The lower limit for dissolved oxygen -- that is, the point at which the dissolved oxygen -- is so reduced

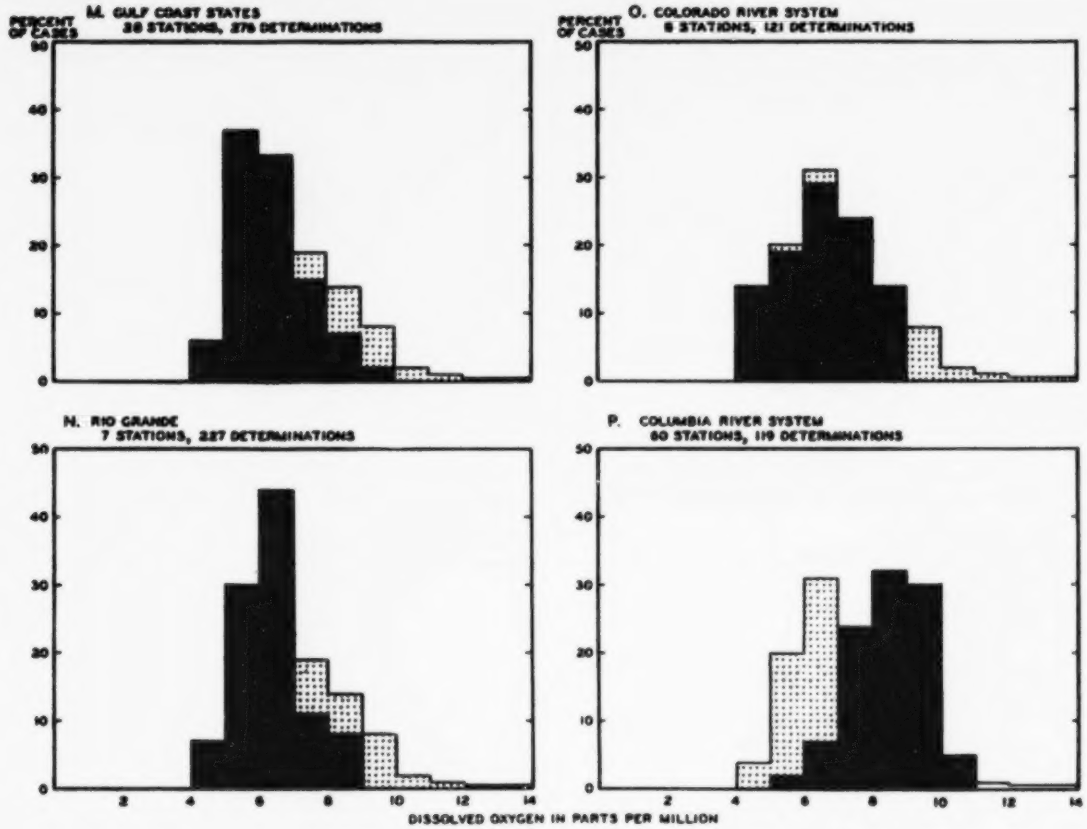


Figure 9 - Continuation of dissolved oxygen comparisons, stippled Graph A in Figure 6, being the standard.

as to present a lethal condition for fishes is equally difficult to define. Kupzis (1901) reports that in general the cyprinid, Leuciscus erythrophthalmus (the European roach), could live for sometime in water containing 0.7 cubic centimeter of dissolved oxygen per liter (1 p.p.m.) but that this species of fish died from asphyxia when the dissolved oxygen was reduced to 0.4 to 0.5 cubic centimeter per liter (0.57 - 0.71 p.p.m.). Plehn (1924) states that trout live best in water containing 7 to 8 cubic centimeters of dissolved oxygen per liter (10 - 11.43 p.p.m.), but if the water be warm these fish show some discomfort when the dissolved oxygen is reduced to 5.5 cubic centimeters per liter (7.86 p.p.m.). This author also states that carp live well in water containing 5 cubic centimeters dissolved oxygen per liter (7.1 p.p.m.), but show respiratory difficulties when the dissolved oxygen is reduced to 3 cubic centimeters per liter (4.3 p.p.m.) or lower. If the water be cold, carp can live for a short time in water containing only 0.5 cubic centimeter per liter of dissolved oxygen (0.71 p.p.m.). In order of their oxygen requirements, Plehn lists first the salmonids and coregonids, then the barbe, the rutte, the pike, carp, tench, goldfish, and lowest of all the eel. Gardiner and King (1922) give asphyxial point for trout as from 0.8 cubic centimeter of dissolved oxygen per liter (1.14 p.p.m.) at 6.5° C., to 2.4 cubic centimeters per liter (3.4 p.p.m.) at 25° C.; and for goldfish as 0.39 cubic centimeter per liter (0.56 p.p.m.) at 11° C., and 0.42 cubic centimeter per liter (0.6 p.p.m.) at 27° C. Paton (1904) found that harmful to fatal conditions for young trout developed if the dissolved oxygen were reduced to 2 cubic centimeters per liter (2.9 p.p.m.) or lower, although some individuals were able to live in such waters for long periods of time.

Thompson (1925) states that carp and buffalo have been found living in water carrying as little as 2.2 p.p.m. of dissolved oxygen. As a rule, he found a variety of fishes only when 4 p.p.m., dissolved oxygen were present, and the greatest variety of fishes were taken from waters carrying 9 p.p.m. of dissolved oxygen. His observations made at Peoria Narrows, Ill., in the summer of 1923 showed that fishes died over night in waters having less than 2 p.p.m. of dissolved oxygen. Of the various species of Illinois fish discussed by Thompson the dogfish, Amia calva, seemed to be the most sensitive to low oxygen tensions and the carp, the most resistant.

From the various observations cited above, which may be taken as representative of the voluminous literature on the oxygen requirements of fishes, it may be seen that the upper limit of dissolved oxygen at which asphyxia may be expected in fresh-water fishes if there be no unusual complicating factors, is in general about 3 p.p.m. at 25° C. In evaluating the dissolved oxygen level as a lethal hazard to fish life, this upper asphyxial limit must be considered rather than the lower minimal limit of dissolved oxygen which can be tolerated by some fish for varying periods, particularly under the conditions of rapid or abrupt oxygen reduction presented in so many of the experimental studies, since the actual hazard to fish life begins at the oxygen level where death may be expected even if some more hardy individuals survive temporarily or for some time at lower oxygen levels.

It is well established for many animals and man, however, that the asphyxial oxygen level, the oxygen level which will support life if profound compensations be made, and the oxygen level at which respiratory, cardio-vascular, and other systemic compensations begin are three quite different values. The latter -- that is, the oxygen level at which respiratory and circulatory compensations are initiated -- marks the lower limit as regards oxygen percentage of the favorable respiratory environment, although this oxygen level is much higher than the lethal oxygen level for these same species. It has been shown (Ellis, 1919) that although man lives in air normally containing 21-percent oxygen, and the collapse point for most human beings is reached when the oxygen in the air breathed is reduced to approximately 6 percent, that human respiratory compensation to reduced oxygen begins at about 18 percent oxygen. Applying this same principle to fishes in the present studies it has been found that individual goldfish, perch, catfish, and other species of fresh-water fishes in good condition and from favorable environments if placed in water of constant flow, favorable composition, and temperature (20° to 25° C.) may show respiratory compensations in rate and volume or both when the dissolved oxygen in this water is reduced only a little below 5 p.p.m. As there are various factors influencing the exact point at which this respiratory compensation begins, the details will be presented elsewhere; but the important finding in connection with the present discussion is that even under conditions as favorable as may be met with in fresh-water streams, respiratory compensations by fishes to oxygen reduction may begin when the dissolved oxygen level is still almost 5 p.p.m. From these studies the case of a perch which made respiratory compensation to reduced oxygen largely by rate is presented (Fig. 10) as typical.

This finding that respiratory compensations by fresh-water fishes to oxygen reduction may begin when the dissolved oxygen of water is lowered only to approximately 5 p.p.m. is in accord with the statement of Plehn (1924) that carp show respiratory difficulties when the dissolved oxygen is reduced to 4.3 p.p.m. and gives physiological background for the differences in fish faunae between waters carrying 4 p.p.m. or less dissolved oxygen and those carrying 5 p.p.m. or more dissolved oxygen, as reported in our field studies.

Considering the data from all sources and particularly from the field and laboratory studies presented here 5 p.p.m. of dissolved oxygen seems the lowest value which may reasonably be expected to maintain in good condition varied fish faunae of warm-water fishes in our inland streams, if the water temperature be 20° C. or above. This statement does not mean that 5 p.p.m. dissolved oxygen is the lethal point for fresh-water fishes but designates 5 p.p.m. as approximately the lower limit of favorable conditions. The fact that fish on occasion can tolerate for a period of hours or even days water carrying less than 5 p.p.m. dissolved oxygen does not justify the acceptance of such conditions as defining any stream as suitable for fishes, in view of the data presented on the natural preference by fresh-water fishes for waters containing 5 p.p.m. or more of dissolved oxygen, and the experimental evidence from various sources that vital compensations may be called for in waters carrying less than 5 p.p.m. dissolved oxygen at temperatures of 20° C. or above. Besides it is recognized in the various fields of animal husbandry, including aquiculture and pisciculture, that if any variety of animal is to be successful and thrive merely sublethal conditions are not adequate.

The application of this observation concerning sublethal conditions is particularly important in pollution investigations, for a reduction of dissolved oxygen increases the lethality of many stream pollutants, especially those injurious to the gills (v.i.). For example, it was found that certain concentrations of various metallic salts and acid wastes were consistently more injurious to fish when the dissolved oxygen contained in the water thus polluted was comparatively low, but still sublethal, than when the dissolved oxygen was high. This difference in lethality correlated with dissolved oxygen obtained in spite of the fact that these specific pollutants neither reacted with nor were removed by dissolved oxygen regardless of the amount of oxygen present.

The dissolved oxygen content of unpolluted streams normally varies with at least four major sets of factors, namely, (a) physical conditions such as stream flow, stream fall, and temperature, which influence the saturation of water with oxygen from the air; (b) oxygen produced by aquatic plants; (c) oxygen removed by aquatic organisms both plant and animal; and (d) the oxygen demand of the organic detritus in the stream. Effluents polluting streams alter this dissolved oxygen balance chiefly through increasing the oxygen demand. Pollutants may create oxygen demand in any of three ways or combinations of these; that is, by the addition of quantities of organic matter of a putrescible sort, among which may be mentioned domestic sewage, packing-plant wastes, beet-sugar waste, and hide vat liquor from tanneries; through the action of various reducing chemicals as certain sulphite wastes from paper mills, sulphide and iron wastes from mines, and certain spent dyes from leather works; and by killing large masses of aquatic vegetation which subsequently decompose. Many effluents which create oxygen demand are also harmful to aquatic organisms because of specific toxic effects.

The determination of the biochemical oxygen demand of various effluents affecting the dissolved oxygen balance in the stream does not necessarily, therefore, give a true evaluation of the pollution hazards produced by these effluents; for various species

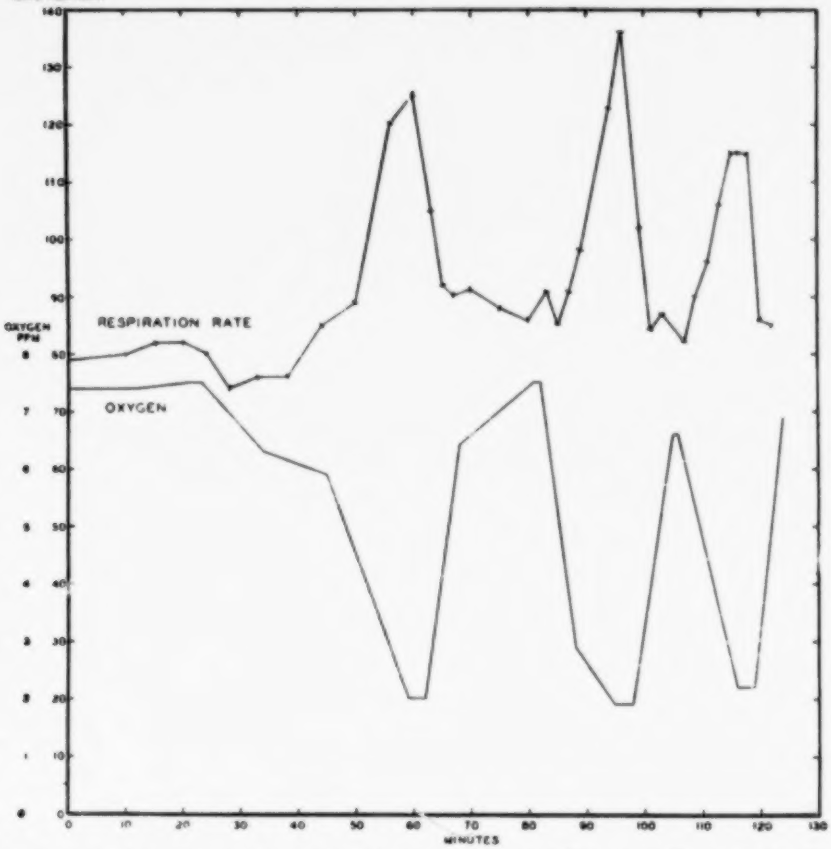


Figure 10 - Changes in the respiration rate of a 4-1/2 inch yellow perch, Perca flavescens, correlated with changes in the dissolved oxygen content of the surrounding water, temperature 18 degrees centigrade.

of warm-water fishes will live in water having high oxygen demand, due to domestic sewage or industrial wastes rich in organic material but without specific toxic substances, if the dissolved oxygen level be maintained above 5 p.p.m. and the aeration be sufficient to blow off the excess of other gases as carbon dioxide, methane, and sulphur derivatives. The biochemical oxygen demands of such effluents as have high oxygen demands must be taken into account, however, if these wastes are poured into streams, in order to compute the dilution required to prevent this oxygen demand from lowering the dissolved oxygen in the stream to an unfavorable level.

Two examples of the effect of dissolved oxygen on the lethality of effluents free from specific toxic substances but presenting large pollution hazards because of high oxygen demands will suffice. The tan vat liquor from a tannery on the upper Mississippi River in spite of the high oxygen demand of this waste was not only tolerated readily when properly aerated and unmixed with the toxic chemical effluents from the plant by various warm water fishes but materially increased the production of plankton when added to the river water. Findings from the sewage treatment system of the city of Munich, Germany, also demonstrate that high biochemical oxygen demand in itself need not be a pollution hazard to fresh-water fishes. In this system, water from the river Isar is mixed with prepared sewage and impounded for the commercial raising of rainbow trout and carp. This procedure was very successful over a period of years in the production of commercial quantities of both trout and carp. The following statement from a recent English review of the work of this plan (Engineering, 1935) may be quoted in this connection:

" The proportion of fresh water to sewage should be at least 5:1, and the proportion of oxygen not less than 5 cubic centimeters per litre (7.1 p.p.m.). If these conditions are maintained, the micro-organisms will flourish, absorbing and destroying all impurities and providing the food for the completion of the process. In such circumstances the fish will suffer no ill effects, although, as is well known, trout are most susceptible to any impurity. "

The oxygen balance of streams may also be affected by oils which exclude oxygen from the surface of the stream and prevent proper reaeration of the water. Oil is rarely poured into streams in such quantities as to present this difficulty, however. Again sewage and industrial sludges are often particularly harmful in reducing the dissolved oxygen through their biochemical oxygen demands during the winter season when ice covers the stream surface and interferes with the reoxygenation of the water.

HYDROGEN-ION (pH) LIMITS

Hydrogen-ion concentration measurements in the laboratory and aboard Quarterboat 348 were made electrometrically with calomel and quinhydrone electrodes (Leeds, Northrup Co., Philadelphia, type 7701-A1). In the field the Youden apparatus (W. M. Welch Co., Chicago, type 5270) bridging the quinhydrone electrode against standardized phthalate solution was used because of greater portability. When field conditions were such that neither of these two pieces of apparatus were available, the determinations of hydrogen-ion concentrations were made colorimetrically by the method of Gillespie (1920). Sterile tubes, solutions, and stoppers were used in preparing the tube series for the Gillespie sets, which were calibrated against the calomel electrode and kept in a dark box except when measurements were being made. Although fractional values were recorded (i.e., hundredths), in presenting the data the value is given to the nearest tenth pH.

The hydrogen-ion concentration of the inland streams of the United States, southern Canada, and northern Mexico, excepting badly polluted portions of these waters, as seen in a review of some 10,000 readings made during the past 5 years, lies in general between value of pH 6.7 and pH 8.6, with the extreme range (in our data) or pH 6.3 and pH 9.0 in streams for which no specific pollution factor affecting the hydrogen-ion concentration was readily observable. Swamp waters, bog streams, and particularly swamp lakes not infrequently show an acidity between pH 4.5 and pH 6.0, yet at the same time may support mixed fish faunae. In some small western streams and pools containing fishes examined by the writer in North Dakota, Montana, and New Mexico an alkalinity of pH 9.5 was occasionally found in alkali districts or near mineral springs. In such waters small poeciliids and cyprinids were uniformly the dominant fishes.

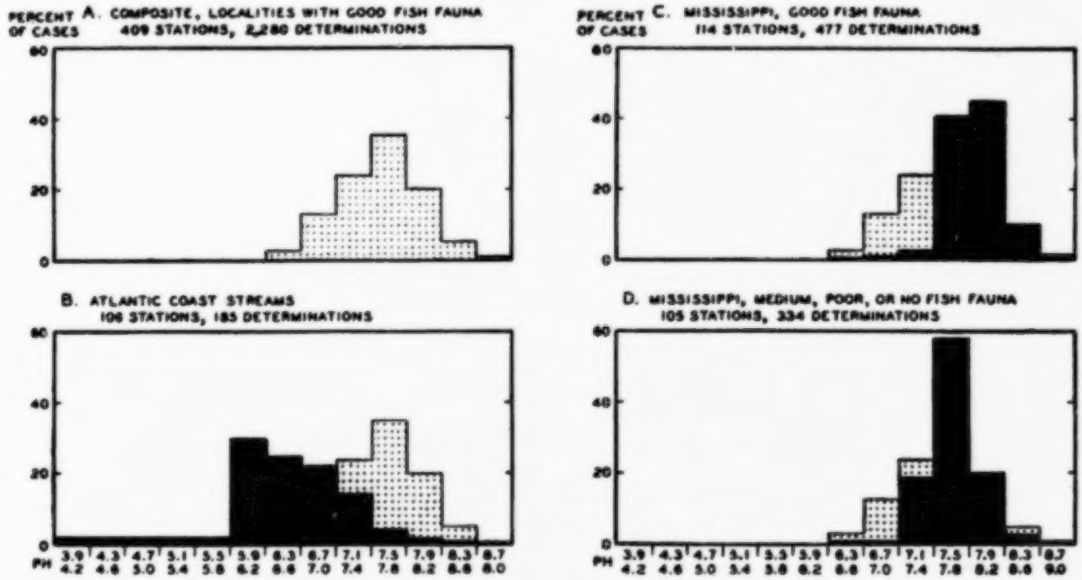
In Figures 11, 12, 13, and 14, 7,228 pH readings from inland stream waters representing 1,125 localities are presented. These data were collected during the warm season, June to September, 1930-35, and are comparable with the dissolved oxygen data in the preceding section. The composite, Figure 11A, was constructed from 2,280 readings representing 409 localities where good fish faunae, as previously defined, were found. This composite covers a range between pH 6.3 and pH 9.0, with 97 percent of the cases between pH 6.7 and pH 8.6. Superimposing the graph of the composite on the graphs presenting the data from the various river systems, however, shows that, except in cases of extreme pollution, the pH values as such of stream waters, both polluted and unpolluted, do not differ materially from those of the composite.

The data from the various river systems (other than the composite) are presented without regard to pollution, except that no pH readings of water in the immediate vicinity of flumes and conduits from which effluents were escaping have been included. The extreme pH range of the flowing waters of inland streams of the United States, both polluted and unpolluted, as found in these field studies was pH 3.9 to pH 9.5, although various effluents poured into these same waters were found to range from pH 1.0 to pH 11.0 at the point of entrance into the stream. These observations show that dilution and the buffer action of various substances in the river waters do change the pH values of the extremely acid and extremely alkaline wastes rather rapidly to the range of the composite pH 6.3 to pH 9.0.

Reviewing all of the field pH data it may be observed within the general range of unpolluted water, pH 6.7 to pH 8.6, that mountain streams, particularly headwater streams, are in general more acid than plains or lowland streams. The Columbia River data (Fig. 14P) bring out this comparison in the bimodal distribution of the pH values, since portions of the Snake River flowing through plains and desert regions and the mountainous headwaters of both the Snake and Columbia Rivers are included in this graph. This same distribution of pH values is also apparent in the graphs presenting the findings in the two parts of the Missouri River system (Figs. 13I and 13J). The explanation of these distributions of pH values is clear when the carbonates and conductivities of these waters are also considered (v.i.), as the headwater streams contain much less dissolved solid matter than the plains, lowland, and desert streams. The effects of acid pollution are evident in the Ohio River (Fig. 13K), and of general pollution and some bog waters in the Atlantic coast streams (Fig. 11B).

The field observations presented here agree in general with the statement made by Shelford (1929) that the hydrogen-ion concentration from pH 6.5 to pH 8.5 may be expected in most uncontaminated fresh-water streams and lakes; and with the observations of Powers (1921 and 1929); and of Juday, Fred, and Wilson (1924).

Experimental tests have demonstrated that many species of fresh-water fishes have a great tolerance for variations in hydrogen-ion concentrations over a wide range. Creaser (1930) showed brook trout to have a voluntary toleration of hydrogen-ion concentrations from pH 4.6 to pH 9.5. Brown and Jewell (1926) found catfish and perch living in apparently good condition in a bog lake, the water of which ranged from pH 4.4 to pH 6.4; and also in a glacial lake nearby, the water of which varied from pH 8.2 to pH 8.7. These workers demonstrated that the fishes from the two lakes survived transfer from either lake to the other. Wiebe (1931a) reports goldfish survive rapid changes from pH 7.2 to pH 9.6; largemouth black bass, from pH 6.1 to pH 9.5; smallmouth black bass, from pH 6.6 to pH 9.3; and sunfish from pH 7.2 to pH 9.6. Powers (1930) in reviewing the problem summarizes the existing data by saying that aquatic organisms are able to withstand a wide range in pH. The writer has

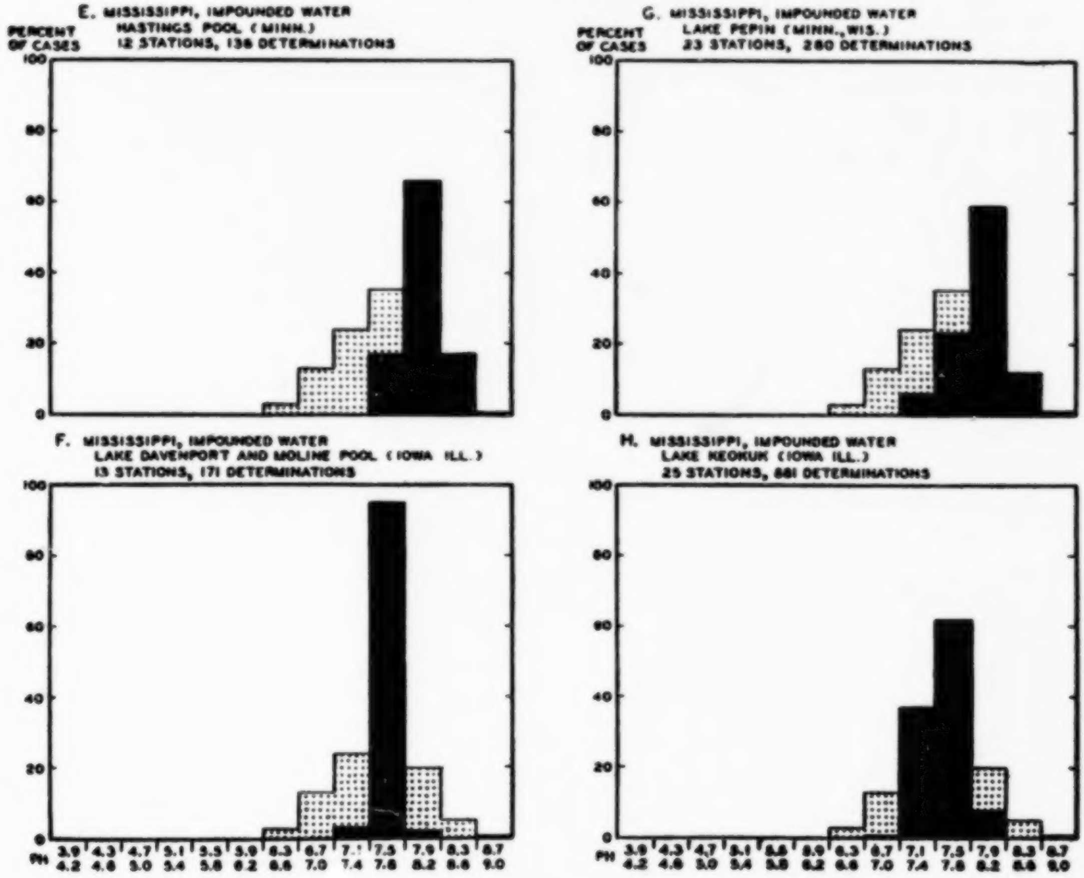

A. COMPOSITE, LOCALITIES WITH GOOD FISH FAUNA 409 STATIONS, 2,280 DETERMINATIONS

B. ATLANTIC COAST STREAMS 106 STATIONS, 185 DETERMINATIONS

C. MISSISSIPPI, GOOD FISH FAUNA 114 STATIONS, 477 DETERMINATIONS

D. MISSISSIPPI, MEDIUM, POOR, OR NO FISH FAUNA 105 STATIONS, 334 DETERMINATIONS

Figure 11 - Comparisons of pH values of various river units. Composite and black graphs as explained under Figure 6.


E. MISSISSIPPI, IMPOUNDED WATER HASTINGS POOL (MINN.) 12 STATIONS, 136 DETERMINATIONS

F. MISSISSIPPI, IMPOUNDED WATER LAKE DAVENPORT AND MOLINE POOL (IOWA ILL.) 13 STATIONS, 171 DETERMINATIONS

G. MISSISSIPPI, IMPOUNDED WATER LAKE PEPIN (MINN.,WIS.) 23 STATIONS, 280 DETERMINATIONS

H. MISSISSIPPI, IMPOUNDED WATER LAKE KEOKUK (IOWA ILL.) 25 STATIONS, 661 DETERMINATIONS

Figure 12 - Continuation of comparisons of pH values for various river units, stippled Graph A Figure 11, being the standard.

confirmed this statement with gammarids, daphnia, unionids, and planaria, as well as for goldfish, perch, and catfish, in connection with pollution tests,

It might seem, therefore, from both the field data and laboratory findings that the pH values of stream water would be of little consequence in pollution studies and in determining standards of water suitability. However, the pH of natural water is determined by substances in solution -- particularly carbonates, carbon dioxide, various salts, and a few organic substances -- which collectively constitute a poor to fair buffer system; so that water more acid than pH 6.7 or more alkaline than pH 8.6 is not found generally in our inland streams, unless there be some unusual factor in the complex. Pond water, bog water, and lake water vary over a wider range, but the combination of stream flow, aeration and buffer substances holds the hydrogen-ion concentration of the larger rivers, the smaller streams, and even many brooks within the limits described. In pollution studies, therefore, it has been found advisable to view with suspicion any stream water having an hydrogen-ion concentration outside of the limits pH 6.7 to pH 8.6, until it could be definitely shown that such extra limital pH values were due to natural causes rather than to human agencies, as even badly polluted streams were usually within these limits. If the water of flowing streams were more acid than pH 6.7 or more alkaline than pH 8.6 as the result of the addition of municipal or industrial effluents the buffer and carbonate systems were usually so disturbed that conditions harmful to fishes were generally found. The determination of pH, therefore, is an important aid in the study of polluted water in spite of the range of tolerance of fishes to pH changes in unpolluted waters, because excessive variation in hydrogen-ion concentrations is indicative of harmful changes in the complex of dissolved substances, both solids and gases, normally found in river water.

Among the effluents which change the pH of stream water and break down the buffer systems are the wastes from wire-nail mills, tin-plate mills, and other metal works where acid washes are used; wastes from chemical works, particularly dye mordant and soda compounds; spent liquors from chrome tanning processes; whey-containing fluids from dairy products

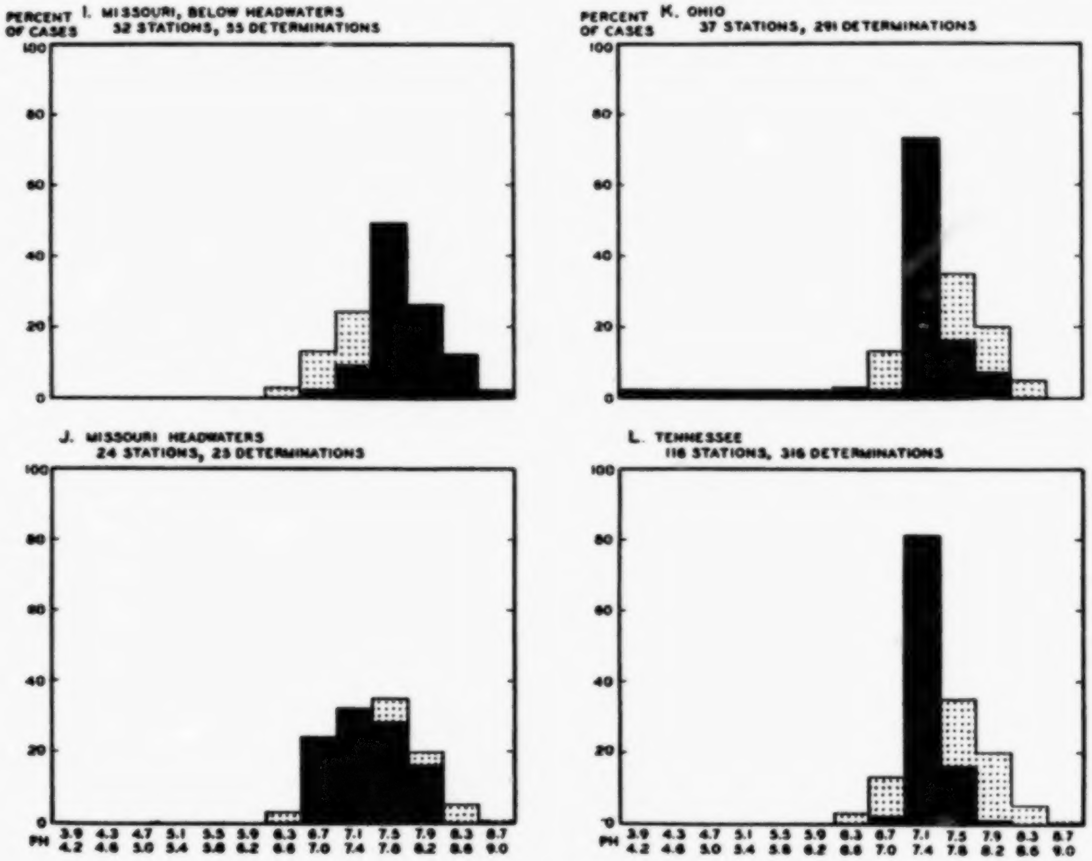


Figure 13 - Continuation of comparisons of pH values, stippled Graph A Figure 11, being the standard.

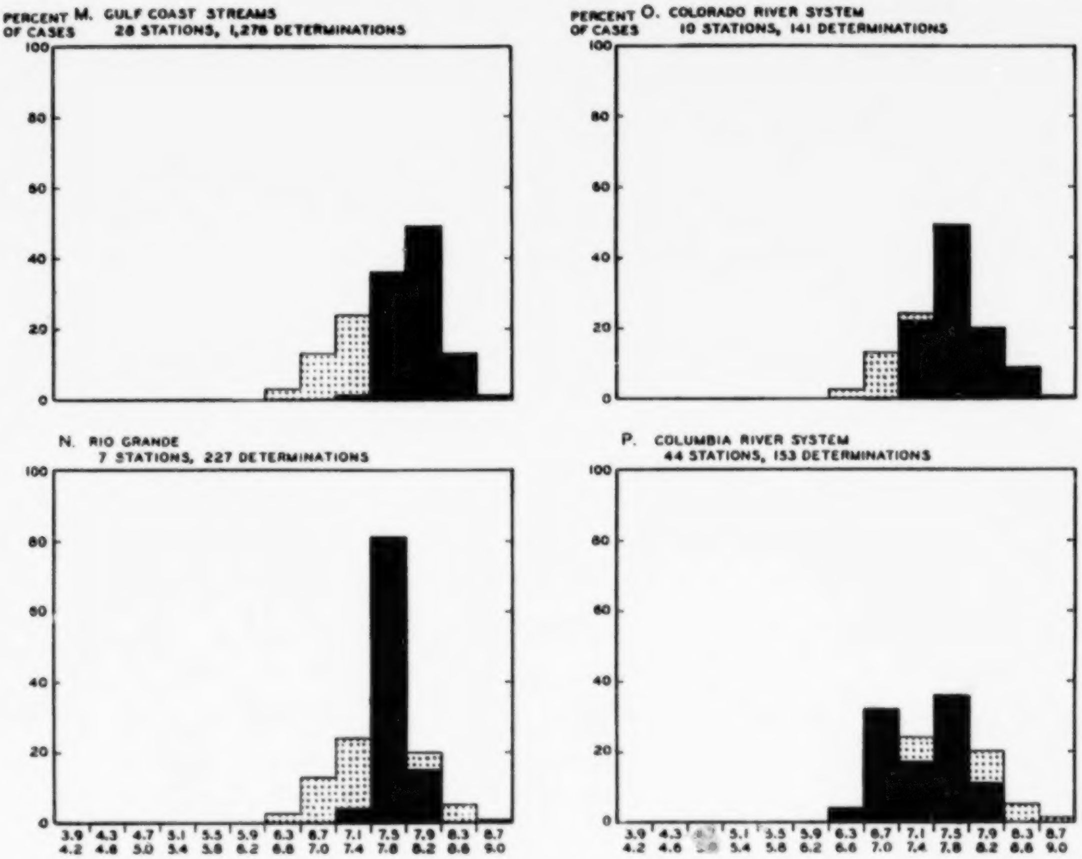


Figure 14 - Continuation of comparisons of pH values, stippled Graph A Figure 11, being the standard.

concerns; laundry waters; and some battery factory wastes. The waters from unsealed coal mines also add large quantities of acid wastes. The specific effects of high and low hydrogen-ion concentrations are discussed in detail in the section on acid pollutants. (See p. 162.)

IONIZABLE SALTS

Specific Conductance

Measurements of specific conductance were made with standard glass cells containing coated platinum electrodes in telephone circuit with a microhummer and standard variable resistance units. This apparatus was found to be very sturdy and was regularly used both in the laboratory and in the field. For convience in presentation, the data are expressed as specific conductance in mho x 10^{-6} at 25° C.

Unpolluted natural waters contain in solution small quantities of carbonates, chlorides, phosphates, and sulphates, usually some nitrates and nitrites if organic matter be present, and traces of many other salts which vary with the region through which the stream flows. The metallic ions represented are largely calcium, magnesium, sodium, potassium, iron, and manganese, with traces of various other elements. Owing to the fact that carbon dioxide is supplied to stream water from so many sources, carbonates are the dominant salts; but because of the low solubility of most carbonates and also of most phosphates, the mineral content of river water never rises very high unless some particular substance is added to the water which will raise the solubility of these compounds or transform them into other more soluble compounds. All of the substances in solution in river water collectively exert osmotic pressure on the aquatic organisms living in the water, and many of those compounds are physiologically active, so that fresh-water fishes and other animals living in these streams have become adapted to the physical and physiological actions of this salt complex.

Small variations in several of these salts may cause small variations in the species composition, particularly the invertebrates, of the faunae at any given station. Most aquatic species, however, will tolerate changes of considerable magnitude in the relative amounts of these salts normally present in flowing waters, if the very small total amount which is usually present be not exceeded. For example, the fixed carbonates in the upper Tennessee River were found to vary from 0.4 to 30 cubic centimeters per liter (computed as CO_2 by volume) without affecting the general composition of the aquatic fauna, and from 0.3 to 55.5 cubic centimeters in Spider Creek, a tributary to the Wabash River.

The specific quantities of most of the substances comprising this salt complex are not so important as the total quantity of soluble matter involved (see section on osmotic pressure), since even the small quantities of these compounds present in ordinary soil run-off are in excess of the physiological needs of most fresh-water organisms. As in general these substances are ionizable, measurements of the specific conductance of a large number of polluted and unpolluted waters were made. The summarized data are presented in Figure 15.

These determinations of specific conductance of inland fresh waters show that, excepting the streams in the plains and desert regions, the specific conductance of those portions of inland streams and rivers which were supporting good, mixed fish faunae in general lay between 150 and 500 mho x 10^{-6} at 25° C.

This uniformity of water composition in flowing streams holds even in the very deep holes of rivers. Two cases -- Pan Eddy, in the Tennessee River, and the deep hole off Grand Tower, Ill., in the Mississippi River -- are presented as typical in Table 1.

Only in the very deep portions of river lakes, such as Lake Wilson in the Tennessee and Elephant Butte Reservoir in the Rio Grande, is there any marked stratification of the waters. In the deeper portions of some of these impounded waters (see Table 4) definite thermal stratification, with the attendant changes in turbidity, dissolved oxygen, pH, and other physiochemical features of the water, develop in midsummer at levels variously determined by the general climatological features of the region and the level and amount of draw-off as made for the needs of industry and navigation. In such river lakes a warm, more turbid stream -- the hyperlimnorrheum -- flows over a colder, clearer lake -- the hypolimnion -- with a rather well-delimited thermocline lying between these. This condition of stratification obtains, however, only during a portion of the warm season. Specific data for one station on Lake Wilson (see Table 4) show such stratification of the waters when it was at its height. During the colder portions of the year there is a rather complete mixing of the waters at all depths, even in Lake Wilson. Details of these hydrobiological data of various river lakes are presented elsewhere.

The specific conductance of mountain stream waters was generally in the lower part of this range, unless excess carbon dioxide were present. However, in the streams of the western plains and desert areas, particularly those carrying the more alkaline waters, as the Snake (Columbia River system), the Rio Grande (below headwaters), the Colorado and Gila Rivers, and the upper Missouri and Yellowstone below the mountains, a specific conductance of 2,000 mho was not unusual, the general range of specific conductance of these streams being between 200 and 2,000 mho, with extreme readings of almost 5,000 mho x 10^{-6} at 25° C. However, good mixed fish faunae of such species as normally inhabit these western streams were usually not found in waters with specific conductance greater than 2,000 mho x 10^{-6} at 25° C. These high conductivity values for certain western waters were clearly due to the natural high solids content of these waters which drain areas rich in the various soluble alkalis.

From the field studies on specific conductance it was found advisable in pollution studies to look for specific pollutant action if the conductivity of the water exceeded 1,000 mho in all types of streams except those draining the more alkaline regions, or 2,000 mho in the case of the western streams discussed above, for the specific conductance is very readily raised by acid and salt pollutions of several sorts.

Specific conductance, therefore, offers a ready method for detection of salt and acid pollution, as produced by water from oil wells, by wastes from industries using salts or strong acids, and by soluble salts of heavy metals. This method was used very satisfactorily by the writer in determining the extent of downstream pollution from lead and zinc mines in the Coeur d'Alene district in Idaho (Ellis, 1932), and in following pollution by acid mine waters from coal mines in Tennessee and West Virginia.

CARBON DIOXIDE

Determinations of free, half-bound and fixed carbon dioxide were made by the methods of Seyler as described by Kemmerer, Bovard, and Boorman (1923) and American Public Health Association (1933). The data are expressed as cubic centimeters of carbon dioxide per liter.

FIXED CARBON DIOXIDE (CHIEFLY CALCIUM AND MAGNESIUM CARBONATES)

In Figures 16 and 17 the data from 6,770 determinations of fixed carbon dioxide in flowing streams of the United States and southern Canada collected during the warm season, June to September, inclusive, 1930-35, are presented. The extreme range of fixed carbonates varied from almost 0 to 70 cubic centimeters per liter, with 40 cubic centimeters per liter as the usual upper limit. A composite of determinations from waters where good fish faunae were thriving (Fig. 16A) covered the entire range from 0 to 70 cubic centimeters per liter. In this composite 96 percent of the 2,190 analyses showed the stream water to carry less than 50 cubic centimeters per liter of fixed carbon dioxide and 53 percent lay between 10 and 30 cubic centimeters per liter. Comparing these findings with the classification of lake water given by Birge and Juday (1911), it may be seen that most of the flowing waters fell in the second class, designated as medium waters; and that the large percent of the river and stream waters could be classified as either soft or medium. Hard waters were distinctly in the minority.

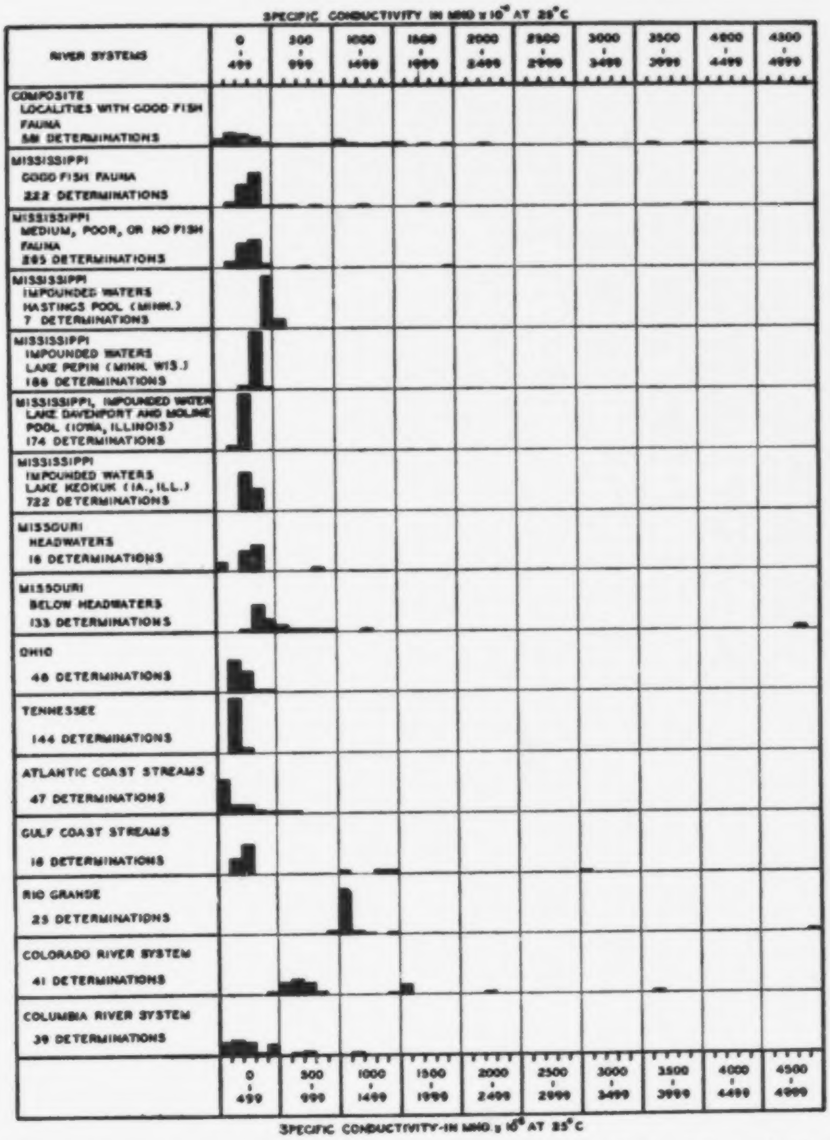


Figure 15 - A comparison of the specific conductivity values of the waters of various river units.

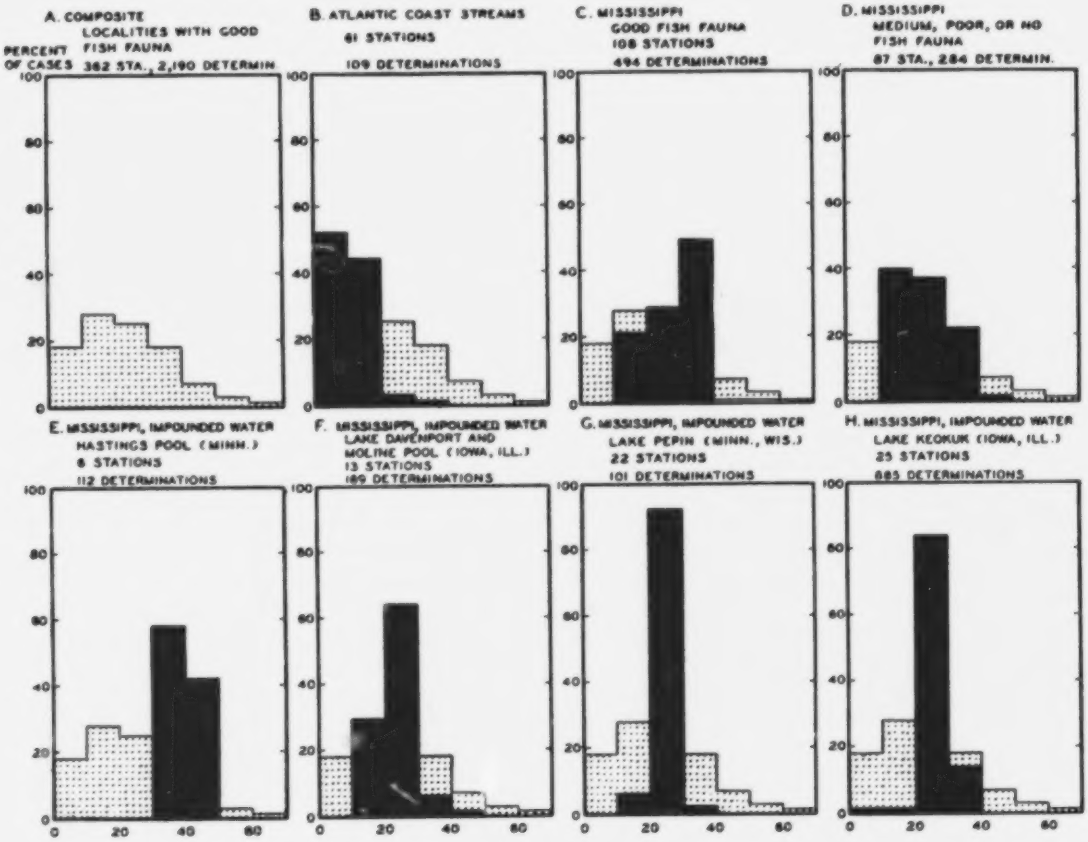


Figure 16 - Fixed carbon dioxide values (as cubic centimeters of carbon dioxide per liter, bottom line) for the waters of various river units. Stippled and black graphs composed as explained under Figure 6.

Reviewing this composite in connection with the findings on fixed carbonates in the waters of the various river systems, it is evident that between 10 and 40 cubic centimeters of fixed carbon dioxide may be expected in most of the fresh-water streams of the United States. Fixed carbon dioxide in itself, however, does not seem to be a determining factor in classifying flowing waters as suitable or unsuitable for fresh-water fishes. Various limnologists have pointed out the importance of fixed carbonates in maintaining food supply, and, therefore, fish faunae of various lakes (Welch, 1935), but from the data on rivers and streams presented here it must be concluded that either carbonates are not limiting factors or that they are always present in sufficient quantities to meet the needs of the stream biota. An exception to this last statement, however, must be taken at least in the case of fresh-water mussels, as carbonates do constitute a very vital limiting factor for these animals.

In general, it was found that the fixed carbonates vary with the character of the country drained, mountain streams in regions where igneous rocks dominate being very low in fixed carbonates and streams traversing limestone country or alkaline soil being relatively high. However, it must be added that excepting the headwater streams in mountainous regions the fixed carbonate load of flowing stream water was rarely less than 5 cubic centimeters per liter.

In pollution studies, although fixed carbonates may not be important in defining particular water as suitable or unsuitable for good fish faunae, the fixed carbon dioxide is of large importance in evaluating the extent of certain types of pollution and in determining the amount of dilution required to render certain effluents relatively harmless to aquatic life. The fixed carbon dioxide of the stream water is the major buffer against which both acid and alkali pollutants act, and the fixed carbonate must be taken into consideration, therefore, when the maximal nontoxic dilution of any effluent is to be determined. This problem is discussed more fully under acid wastes, but it may be pointed out here that the toxicity to fishes and other aquatic life of many of the common acids is much greater in distilled water or water carrying very little fixed carbon dioxide than in water carrying 20 to 60 cubic centimeters per liter of fixed carbon dioxide. The

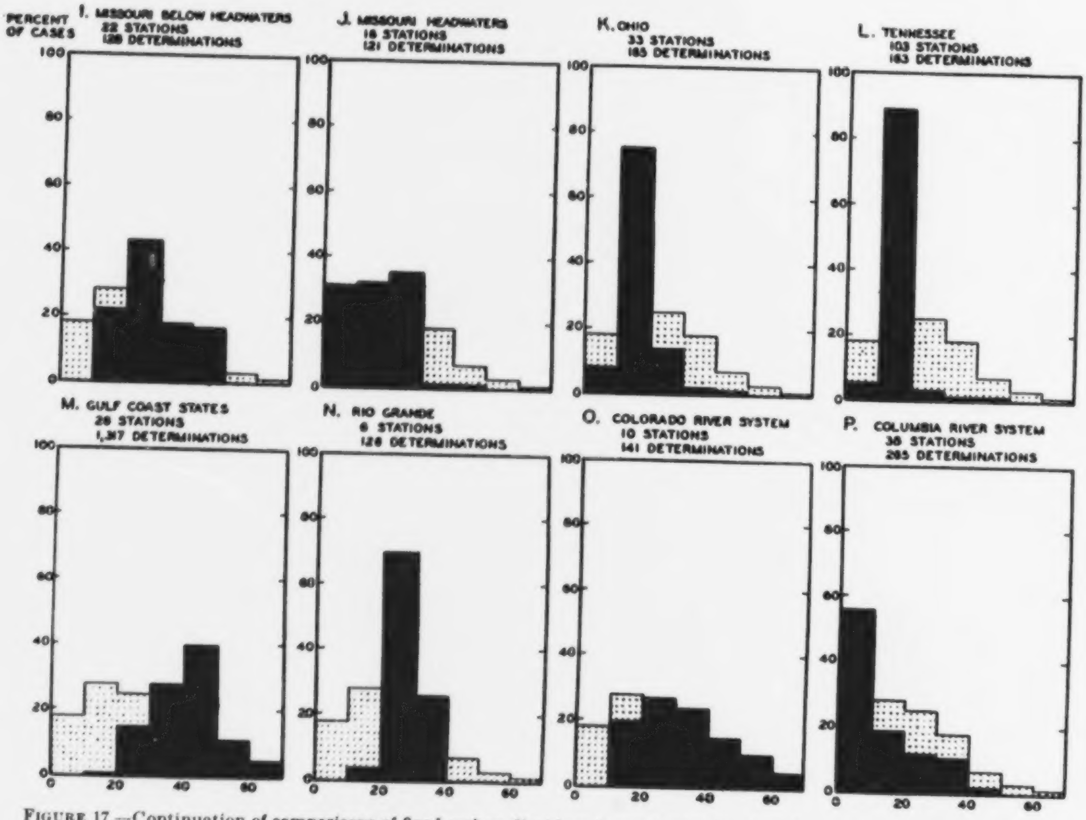


FIGURE 17.—Continuation of comparisons of fixed carbon dioxide values, stippled graph A figure 16 being the standard.

Figure 17 - Continuation of comparison of fixed carbon dioxide values, stippled Graph A Figure 16 being the standard.

reason is obvious because of the buffer and neutralizing actions of these fixed carbonates on free acids. Again, the fixed carbon dioxide is of considerable importance in detoxifying various effluents, particularly those carrying salts of heavy metals, by precipitating various compounds, since many carbonates are relatively insoluble and, therefore, are removed at least temporarily from the waters during precipitation. This is discussed more fully in the section on metallic poisons.

FREE CARBON DIOXIDE

Figures 18 and 19 present the data from 3,351 determinations of free carbon dioxide in various streams of United States. From these figures, and particularly from the graph of the composite, it may be seen that river and stream waters, where good fish faunae were found, carried consistantly less than 5 cubic centimeters of free carbon dioxide per liter, 90 percent of the waters where good fish faunae were taken carrying less than 2 cubic centimeters of free carbon dioxide per liter. It must be pointed out in this connection that conditions in moving streams are quite different as regards free carbon dioxide from those obtaining in lakes and ponds. Due to the constant turnover of river and stream waters because of current action, there is a very uniform mixing of water from surface to bottom (see data from Grand Tower on the Mississippi and Pan Eddy in the Tennessee (Table 1)), so that the free carbon dioxide, as well as the other dissolved gases, is quite uniformly distributed throughout the stream. Again, the movement of the current facilitates the constant reaeration of stream waters with a tendency for these waters to lose carbon dioxide to the air as soon as the carbon-dioxide tension in the water exceeds that of the surrounding air, which is usually quite low (2 to 4 parts per 10,000). Both of these conditions, together with the usual presence of calcium and magnesium salts in stream waters, tend to keep these flowing streams more alkaline than pond, lake, and bog waters, so that in many river waters the alkalinity is raised to a point where there is little or no uncombined carbon dioxide.

The specific toxicity of carbon dioxide for aquatic organisms is well known and is discussed under acid wastes. From all of the field data and in view of the

TABLE 1.—*Characteristics of water at different depths in deep holes of flowing rivers, showing general uniformity of composition at any given station due to mixing action of current*

I. UPPER TENNESSEE RIVER, AT PAN EDDY, 18 MILES BELOW CHATTANOOGA, TENN., AUG. 21, 1931, AIR TEMPERATURE, 23° C.

Depth in feet	Water temperature, degrees centigrade	pH	Specific conductivity in mho × 10^{-6} at 25° C.	Fixed carbon dioxide, cubic centimeters per liter	Dissolved oxygen parts per million	Light penetration, millionth intensity depth in millimeters
S[1]	28.0	7.3	169	15.4	5.6	544
10	28.0	7.2	169	15.4	5.5	561
20	27.9	7.3	169	14.9	5.7	561
30	27.9	7.3	169	14.9	5.9	561
40	27.8	7.3	169	14.9	6.0	561
50	27.8	7.3	169	14.9	5.7	561
60	27.8	7.3	169	14.9	5.2	561
70	27.8	7.4	169	15.2	5.5	552
80	27.8	7.4	169	15.2	5.7	552
90	27.8	7.4	169	15.2	5.5	544
100	27.6	7.4	169	15.4	5.6	536
110	27.7	7.3	168	14.9	5.7	528
120	27.8	7.3	168	14.9	6.1	521
130	27.8	7.3	167	15.1	5.9	513

II. MISSISSIPPI RIVER, OFF TOWER ROCK, NEAR GRAND TOWER, ILL., SEPT. 8, 1931, AIR TEMPERATURE 30.5° C.

S[1]	26.2	7.5	340	26.4	5.3	129
10	26.0	7.5	341	26.6	5.1	125
20	25.8	7.6	339	26.8	5.3	125
30	26.0	7.5	337	26.4	5.3	122
40	26.0	7.5	338	26.4	5.3	129
50	26.0	7.5	340	26.4	5.3	120
60	26.0	7.5	331	26.6	5.3	122
70	26.2	7.4	338	26.7	5.3	122
80	26.0	7.4	342	26.7	5.3	125
90	26.0	7.4	339	26.8	5.4	122
100	26.0	7.4	338	26.8	5.4	119
110	26.0	7.4	341	26.8	5.3	124

[1] Surface, i. e., top 12 inches of water.

experimental findings presented, the determination of free carbon dioxide was found to be a valuable aid in pollution studies, and river and stream waters carrying more than 3 cubic centimeters of free carbon dioxide per liter were checked carefully for some source of organic pollution. Values in excess of 3 cubic centimeters per liter usually were indicative of such pollution in our flowing inland streams. The relatively high free carbon dioxide as compared with the composite found in Hastings Pool in the upper Mississippi River and in the polluted portions of the Atlantic coast streams and other systems are evident from the graphs presented in Figs. 18E and 18B.

IRON

Iron determinations were made by the method of American Public Health Association (1933), using permanent standards of cobalt and platinum salts.

A limited number of total iron determinations were made in typical localities for correlation with studies of water from abandoned coal mines. Eighty-three determinations of total iron in waters of Mississippi, Missouri, Ohio, Tennessee, Atlantic coast, and Gulf coast systems gave the following grouping:

At stations where good fish faunae were found (75 cases) the free iron ranged from a trace to 30 p.p.m., with 69 of these 75 cases below 10 p.p.m. In streams polluted with either mine waters or chemical effluents carrying iron compounds the total iron ran much higher, the maximum found in this series being 202 p.p.m. in waters of a stream of high acidity (pH 4.4) flowing from a coal mine in operation near Turley, Tenn. Iron in quantities above 100 p.p.m. was associated with acidity above average and when such conditions were found chemical or mine pollutions were suspected.

In the experimental tests (see section on heavy metals) it was found that 100 p.p.m. of total iron were not immediately harmful to either fish or daphnia; and that such quantities, unless a constant flow were maintained, were usually diminished during the first few days of experimental tests as a result of precipitation so that the free iron in the water did not exceed 20 p.p.m. Iron as it occurs in most natural flowing waters, therefore, is not a major limiting factor in the distribution of fishes in fresh-water streams. Besides in fresh-water streams and rivers the constant reaeration of water readily offsets any important oxygen loss through iron compounds, as has been reported from bog lakes and certain deep lakes where large quantities of iron were found.

In pollution studies of the inland fresh-water streams, therefore, the determination of total iron is helpful in locating the source of acid in highly acid waters, as a combination of high iron and high acidity suggest the source of acidity as pyrite or coal beds if

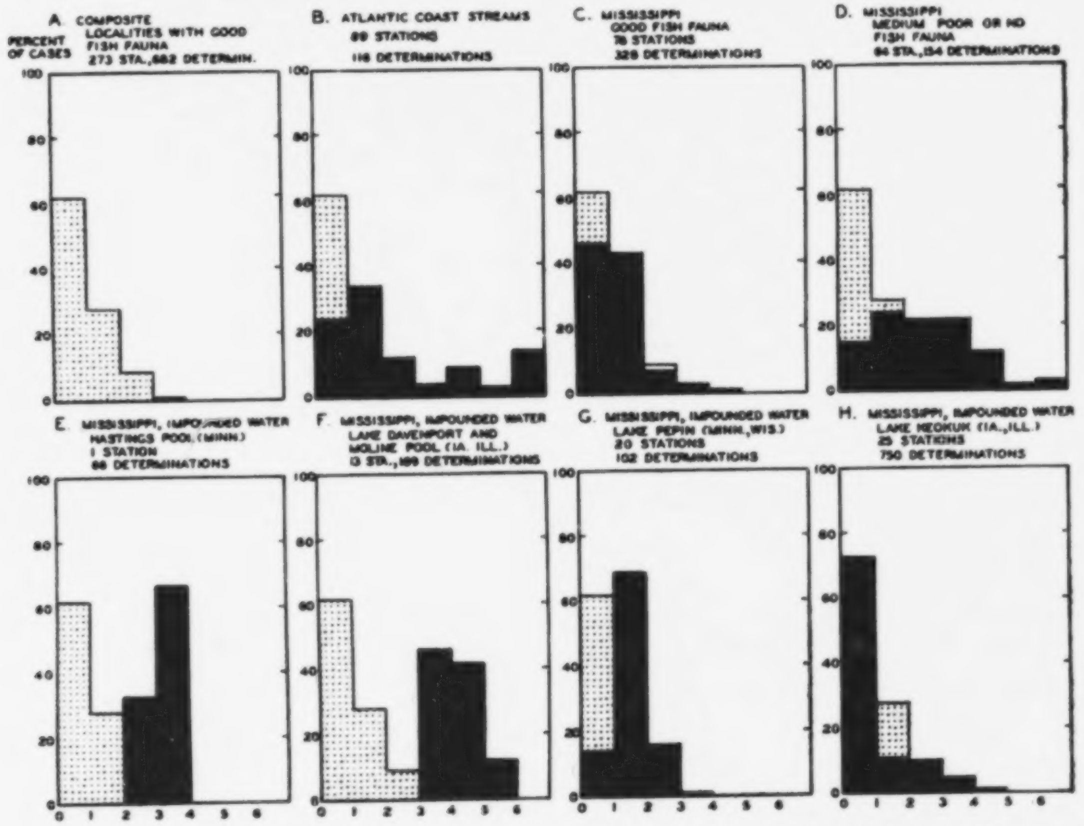


Figure 18 - Free carbon dioxide values (as cubic centimeters of carbon dioxide per liter, bottom line) for the waters of various river units. Stippled and black graphs composed as explained under Figure 6.

no specific chemical effluent is responsible for these conditions.

AMMONIA

Total ammonia determinations were made by the Nessler method as described by American Public Health Association (1933).

Decomposing organic matter, if nitrogenous, will liberate into stream water, ammonium compounds representing a considerable portion of the total nitrogen. As ammonium compounds present at once both a hazard due to the high toxicity of ammonium carbonate for most aquatic animals, and an important source of nitrogen for the lower plants in the aquatic food chain, ammonia determinations give a significant index of the balance between stream purification through the consumption and elimination of ammonium compounds formed during the disintegration of organic detritus, and the amount of such organic wastes received by the stream. In natural unpolluted waters organic detritus consists primarily of the remains of the organisms, both plant and animal, dying in the stream and adjacent waters, together with such organic matter as may be brought into the stream by surface run-off water. In unpolluted water, therefore, the amount of ammonia and ammonium compounds (chiefly ammonium carbonate) is usually very small.

The West Riding Rivers Board (1930) found the ammonia content of the River Wharfe, an unpolluted stream, to vary between 0.0 and 0.17 p.p.m.; Butcher, Pentelow, and Woodley (1927) state the ammonia content of the river Itchen, normally to be less than 0.1 p.p.m. with a maximum of 0.25 p.p.m.; Pearsall (1930) found the waters of various English lakes to carry less than 0.01 p.p.m.; Domogalla, Juday, and Peterson (1925) report the ammonia in Lake Mendota to vary between 0.0073 and 0.76 p.p.m.; and unpolluted portions of Wisconsin River above Rhinelander, Wis. (Wisconsin State Board of Health, 1927) averaged 0.096 p.p.m. ammonia. In field studies by the Columbia, Mo., unit similar ammonia values, all below 0.9 p.p.m., were obtained from analyses of unpolluted flowing stream waters.

Polluted streams, even if carrying only a very small load of organic wastes, present quite a different picture and, as Winslow and Phelps (1906) have

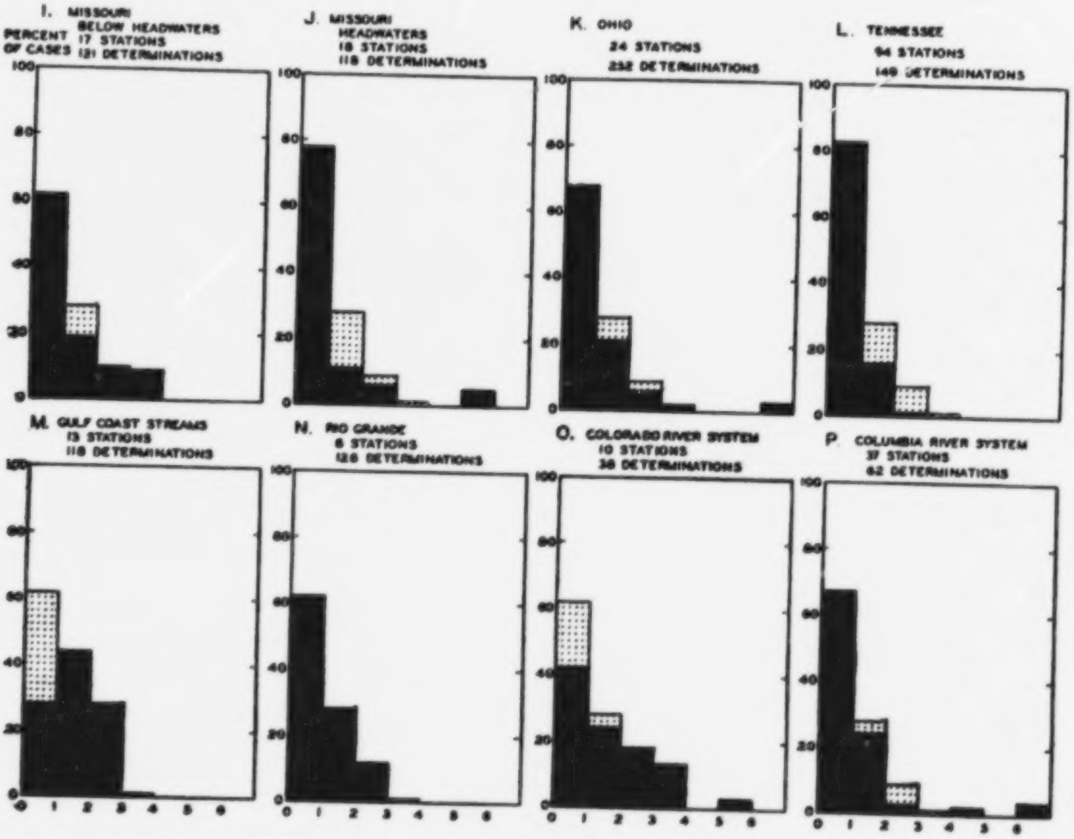


Figure 19 - Continuation of comparisons of free carbon dioxide values, stippled Graph A Figure 18, being the standard.

pointed out, from one-third to one-half of the total nitrogen of sewage will be in the form of free ammonia, largely as ammonium carbonate; and sewage will carry from 15 to 35 p.p.m. or more of total nitrogen. Wiebe (1931b) reported a maximum of 0.224 p.p.m. ammonia in the Mississippi River at Fairport, Iowa. Ellis (1935b) found from 0.36 to 1.16 p.p.m. ammonia in the Mississippi River at Davenport, Iowa, during low waters in the month of July (1934) in portions of the river that were not badly polluted and which were at the time supporting bass, catfish, and other warm-water fishes; and from 0.24 to 3.80 p.p.m. of ammonia in the badly polluted waters of the Mississippi River during September 1935 between St. Louis, Mo., and Cairo, Ill. In the highly polluted Blackstone River, the Massachusetts State Board of Health (1913) reported 11.7 p.p.m. of ammonia. Similarly the writer has found 5.68 p.p.m. of ammonia in the Cache la Poudre River in Colorado at a point where there was heavy pollution with beet sugar factory wastes.

In Table 2 a group of ammonia data typical of the field findings by the Columbia, Mo., unit are presented. These data include no determinations from the immediate vicinity of sewers or other outlets pouring organic wastes into the streams, and no determinations from restricted local portions of streams in which unusual organic pollution was found. These data show the general range of dissolved ammonia which may be expected in our flowing streams during the warm season, and under the usual conditions of municipal and industrial pollutions.

The composite series includes the ammonia values from all these stations collectively at which good fish faunae as previously defined were taken, and the Mississippi and coastal series include all stations in these systems regardless of fish faunae.

It may be seen that excepting the restricted local areas where heavy pollution was found (data not presented in this table) the dissolved ammonia in flowing streams in general lies below 3 p.p.m. This is due to a variety of physical and chemical factors, chief among which is the constant turn-over of the moving stream waters. The good fish faunae showed a preference to waters containing less than 2 p.p.m. dissolved ammonia, as 79 percent of the cases of this group were found in water containing less than 1.9 p.p.m. dissolved ammonia. The remaining 21 percent were apparently thriving in waters containing between 2 and 3 p.p.m. dissolved ammonia, with one case (the

TABLE 2.—*Dissolved ammonia in various stream waters during the warm season, June to October, inclusive, 1934*

[For convenience of comparison, data are expressed as percents of total number of determinations for each stream unit]

River system	Number of stations	Number of determinations	Dissolved ammonia in parts per million						
			0.0-0.9	1.0-1.9	2.0-2.9	3.0-3.9	4.0-4.9	5.0-5.9	Greater than 6.0
Composite [1]	72	178	21	58	21	(2)	--------	--------	--------
Entire Mississippi system	182	478	24	49	22	3	2	(2)	(2)
Coastal streams	58	91	7	55	33	1	1	1	--------

[1] Derived from ammonia determinations at 72 stations throughout the entire Mississippi system and in coastal streams, at each of which stations good mixed fish faunae and associated organisms were thriving.

[2] Less than 0.5 percent.

maximal indicated by footnote 2 in the 3 - 3.9 column) in waters carrying 3.5 p.p.m. dissolved ammonia. This maximal dissolved ammonia value among the stations where good fish faunae were taken was found at Reeds Landing, Minn., where the Mississippi River was flowing rapidly over a sandy bottom at a point just below extensive beds of aquatic vegetation in the foot of Lake Pepin. It must be noted that without exception those waters carrying 2 to 3 p.p.m. ammonia and at the same time supporting the good fish fauna were high in dissolved oxygen; that is, 5.5 to 7 p.p.m., were of low turbidity, and were flowing over good bottoms. Toleration of dissolved ammonia above 2 p.p.m. under field conditions was always associated with otherwise good to exceptionally favorable conditions.

The toxic effects of ammonia compounds have been the subject of many investigations; and aquatic animals have been shown to be particularly sensitive to ammonium carbonate, the form in which ammonia is most frequently found in inland waters, (Shelford, 1917; Belding, 1928; Steinmann, 1928; and McCay and Vars, 1931). Thirty p.p.m. of ammonia will kill some tench, trout, and salmon rather rapidly (Weigelt, 1885), and 55 to 77 p.p.m. will kill shiners and carp in a few minutes to a few hours (Clark and Adams, 1913). A review of the literature on ammonia, however, shows that some observers obtained toxic effects with much smaller quantities.

Ellis and Chipman (1936) have repeated many of the earlier tests and extended the observations to daphnia and gammarids, as well as fish, finding that pH is a large factor in regulating the toxicity of ammonium compounds for aquatic animals, ammonium salts becoming more toxic in more alkaline media. This fact explains the relatively high toxicity of ammonium carbonate to aquatic organisms as compared with other ammonium salts. From these data (presented in section on ammonia pollutants) curves were drawn showing that the toxicity of ammonium compounds increases 200 percent or more between pH 7.4 and pH 8.0. The lower limit of toxicity (death in 10 days or less, depending upon conditions of experiments) was found to be near 2.5 p.p.m. of ammonia. Some acclimatization to ammonia is possible, and it is well known that individuals of various species of fish may be found in water containing 3 to 10 p.p.m. of ammonia. However, the existing literature and the data from our experiments indicate that under average stream conditions with pH value 7.4 and pH 8.5, 2.5 p.p.m. of ammonia will be harmful to many individuals at least of the common aquatic species.

Therefore, in view of the small amount of ammonia found in unpolluted natural flowing waters, 1.5 p.p.m. dissolved ammonia was considered the maximal amount of dissolved ammonia not suggestive of specific organic pollution. In flowing streams 2 to 3 p.p.m. were almost always associated with definite organic pollution and values above 3 p.p.m. in our field studies were always traceable to sewage or factory effluents.

SUSPENSOIDS

The amounts of finely divided suspensoids in various waters and light penetration into these waters were determined by a photoelectric apparatus described by Ellis (1934).

The suspensoids -- that is, particulate matter in suspension in inland fresh waters -- consists normally of erosion silt, organic detritus (as discussed under ammonia), bacteria, and plankton. Each component of this mixture, with the exception of plankton, may be greatly augmented by man's agencies, as quantities of powdered rock, cellulose pulp, sawdust, semisolid sewage, and other debris are added to natural waters.

Parts of some streams, as the Yellowstone and Missouri -- draining areas in which natural erosion has been proceeding rapidly -- have been muddy with their loads of erosion silt since before the earliest records by man and have as a result limited fish faunae. In a large proportion of the inland streams, however, erosion silt, organic detritus, and bacteria were formerly in balance over a considerable portion of the year and conditions favorable to aquatic life maintained, although now and then floods and other unusual conditions killed many aquatic animals in these streams by inundations of silt. With the advent of civilized man and unrestricted deforestation, agriculture, and other uses of the earth's surface, the erosion problem has become gigantic, and the effects of the loads of erosion silt carried by these once relatively clear streams overwhelming on aquatic life in many places.

Erosion silt and other suspensoids (disregarding any specific toxic action of suspensoid wastes) affect fisheries directly by covering the bottom of the stream with a blanket of material which kills out the bottom fauna, greatly reduces the available food, and covers nests and spawning grounds; and also by the mechanical and abrasive action of the silt itself which may clog and otherwise injure the gills and respiratory structures of various aquatic forms, including many fishes and mollusks (Ellis, 1936a). The mechanical action of silt and other suspensoids may not be severe or even harmful to the gills and other structures of the free-swimming fish which move about above the bottom and are not mired down by the settling deposits of the suspensoids, if the amounts of the suspensoids in the water are not too great and if the action of the suspensoids is uncomplicated by other pollutants. Normal fish and many other swimming animals secrete continuously quantities of mucus which wash away suspensoid particles as they lodge on the gills and other exposed parts. Consequently, healthy and uninjured fishes can move through very muddy water or water carrying considerable quantities of pulps (Cole, 1935b), sawdust, and other suspensoids and receive little or no mechanical injury to the gills. However, as has been pointed out by Marsson (1911), small amounts of various acids, chemical wastes, and other substances which in themselves either injure the gills or alter the flow of mucus may greatly augment the mechanical action of the suspensoids, with serious results to the fish, so that through the combined action of these chemical agents and the suspensoids the abrasive action on the gills may be increased or the gills matted with deposits which under more favorable conditions would have been washed away by the mucus.

Indirectly, but none the less effectively, erosion silt affects fisheries by screening out the light, by "laking down" organic wastes, and thus increasing the oxygen demand at the bottom of the stream, and by retaining many forms of industrial effluents as oils, chemical wastes, and pulps in beds on the floor of the stream, with disastrous results to the bottom fauna.

Summarized data on turbidity of the light penetration into various stream waters are presented in Table 3.

From the fieldwork on unpolluted streams in areas where surface erosion was not materially influenced by man and where water conditions were otherwise favorable, it has been shown by Ellis (1936a) that the millionth intensity depth (i.e., the level at which the light entering the surface of the stream would be reduced to one-millionth of its surface intensity) for clear unpolluted streams carrying little or no erosion material is 50 meters or more, and that in streams carrying a heavy load of erosion silt, like the Missouri River, the millionth intensity level may be reduced to less than 100 millimeters. Until erosion is brought under control little can be done in demanding a minimum amount of silt and consequently no standard has been suggested here, but data from over 6,000 determinations on inland streams show that the silt load of these streams should be reduced so that the millionth intensity level would not be less than 5 meters, if conditions even approximating those of times past when erosion was held in check by forest and grasslands are to be restored, in the average inland stream of the United States. The detrimental nature of erosion silt as regards fisheries is discussed more fully by Ellis (1936a).

However, particulate matter introduced by man into streams can be regulated, and the detrimental action of various types of suspensoid pollutants is discussed under that heading.

DEPTHS

As various depths of water are selected by different species of aquatic animals, and even by different ages of the same species in many cases, there is naturally no single optimum depth of stream water for all types of aquatic life. Depth studies of streams and flowing waters, however, bring out differences between waters of natural lakes and those of flowing streams and river lakes.

Current action in flowing streams mixes the water so thoroughly and continuously that in general the composition of the aquatic environment as presented by the water itself varies but little from a few inches below the surface to a few inches above the bottom of the stream. The temperature of the water, its turbidity, dissolved solids content, pH, and even dissolved gases content are much the same throughout the bulk of the stream at any given station, if there by definite current action. Sloughs and other rather isolated lateral areas, particularly those containing large masses of aquatic vegetation, are of course excepted, but even in such cut-off portions of streams the actual differences in water characteristics as compared with the main stream mass are often small.

Of significance in pollution studies, these depth data show that pollutants may be expected to mix rather rapidly and completely with the waters in streams and even river lakes unless these river lakes be more than 50 feet deep, and even in those river lakes unless the seasonal conditions are such as to promote the stratification just described in the deeper portions of such lakes; and that the products of bottom pollution will also be distributed throughout the waters of the stream below such pollution.

TEMPERATURE

As the temperature of the water has bearing on several factors associated with the general problems of pollution, such as dissolved oxygen carrying power of the water, the rate of bacterial decomposition of organic pollutants, and the metabolic demands of the aquatic organisms themselves, the temperature range of flowing streams and river lakes during the warm season is presented in Table 5. These data include no headwaters in mountainous regions, and give, therefore, the water temperature range to be expected in the average inland stream of the United States during the summer months, which season as has been pointed

TABLE 3.—*Turbidity of stream waters as measured by light penetration (millionth intensity depth in meters) in various river systems during June to September, inclusive, 1932–35*

[For convenience of comparison, data are expressed as percents of the total number of determinations for each stream unit]

River system	Number of stations	Number of determinations	Light penetration expressed as millionth intensity depth in meters					
			Clear	Cloudy	Turbid	Very turbid	Muddy	Very muddy
			5.00–∞	4.90–1.00	0.90–0.50	0.49–0.30	0.29–0.15	0.14–0
Composite, streams supporting good mixed fish fauna[1]	202	514	35	24	17	9	9	6
Mississippi, flowing streams, good fish fauna	52	237	4	11	8	16	25	36
Mississippi, flowing streams, medium, poor, or no fish fauna	79	260	1	18	11	14	38	18
Mississippi, impounded water, Hastings Pool (Minn.)	1	17	....	100	....	....	....	....
Mississippi, impounded water, Lake Pepin (Minn.-Wis.)	12	73	11	88	....	1	....	....
Mississippi, impounded water, Lake Davenport and Moline Pool (Iowa-Ill.)	13	175	....	51	19	19	4	7
Mississippi, impounded water, Lake Keokuk (Iowa-Ill.)	16	571	....	19	28	20	16	17
Missouri, headwaters, clear streams (Mont.-Wyo.)	5	15	....	20	60	20	....	....
Missouri, below headwaters (Mont., Wyo., Colo., Nebr., Kans., Mo.)	19	23	44	31	4	....	4	17
Ohio (Pa., W. Va., Ohio, Ky., Ind., Ill.)	41	82	12	5	15	7	48	13
Tennessee, flowing streams (Ky., Tenn., Miss., Ala.)	93	158	11	8	32	32	17	....
Atlantic-coast streams (Maine to S. C., inclusive)	11	16	36	37	18	....	....	9
Gulf-coast streams (Fla. to Tex.)	11	111	....	18	46	18	....	18
Rio Grande, flowing streams (Tex., N. Mex., Ariz.)	6	25	88	....	4	....	....	8
Colorado, flowing streams (Ariz.-Nev.)	7	36	67	22	8	3	....	....
Columbia, flowing streams (Idaho, Mont., Wash., British Columbia)	17	31	49	30	3	3	3	3

Grand totals: Stations, 585; light-penetration determinations, 2,344

---

[1] Derived from light-penetration determinations made at 202 stations in the Mississippi, Missouri, Tennessee, Atlantic coast' Gulf coast, Rio Grande, Colorado, and Columbia systems, at each of which stations good mixed fish faunae and associated organisms were thriving. Each of these 8 stream units are given equal value in the expression of the composite.

out under the discussion of dissolved oxygen presents certain specific pollution hazards.

Considering the composite in Table 5, it may be seen that in spite of the high air temperatures which prevail in many parts of the United States during the summer months, often for periods of days, stream temperatures above 34° C. are not common, and that 95 percent of the 4,545 cases listed in Table 5 lie below 30° C. On the other hand, 66 percent of these 4,545 cases lie between 22° and 30° C. For comparison with the composite in this table, data from the entire Mississippi system and from the coastal streams of the Gulf States, Texas to Florida, inclusive, are also presented. In the Mississippi system 73 percent of the cases fell between 22° and 30° C., and 68 percent of the cases in the Gulf States have the same range.

In evaluating biochemical oxygen demand, dissolved oxygen content, and specific toxicity values for pollution studies therefore, a water temperature range during the warm season from 15° to 36° C. may be expected in the average inland stream of the United States, with the probability of 66 percent or more that these water temperatures will lie between 22° and 30° C. During the other portions of the year -- that is, outside of the warm season -- the water temperatures of these streams in localities north of the freezing line will fall, with 0° C. as the limit. Our field data also show that south of the usual freezing line -- that is, in streams of southern Texas, Alabama, and Florida -- temperatures as low as 10° C. are not unusual during the midwinter season.

The true headwater streams, particularly those in mountainous regions, present a different temperature range even during the summer months. The maximal temperatures for various streams which could be definitely classified as mountainous headwaters, as found in our field studies, were rarely above 10° to 15° C., with the average values lying around 10° C. or even lower.

BOTTOM CONDITIONS AS AFFECTED BY STREAM POLLUTION

In most stream-pollution studies, conditions at the bottom must be considered, since the bottom of the stream contributes a considerable part of the food of stream fishes through the organisms of the bottom fauna which are either eaten directly by fishes or which are included in the food chains of various species

Table 4.—*Characteristics of water at different depths in river lakes produced by impounding flowing rivers*

I. LAKE WILSON[1]

Depth in feet	Water temperature, degrees centigrade	pH	Specific conductivity in mho x 10⁶ at 25° C.	Fixed carbon dioxide, cubic centimeters per liter	Dissolved oxygen parts per million	Light penetration, millionth intensity depth in millimeters
S[3]	30.2	7.4	177	13.4	7.5	2,053
10	30.2	7.6	178	12.9	7.3	2,053
20	29.5	7.4	181	13.2	6.1	1,297
30	29.0	7.5	178	12.9	5.8	1,075
40	28.6	7.5	175	12.9	5.7	1,075
50	28.2	7.5	173	12.9	5.1	972
60	27.8	7.3	161	12.9	3.6	1,075
70	22.9	7.2	167	15.4	.2	9,036
80	21.9	7.2	158	15.0	.2	9,036
90	21.5	7.1	159	15.0	.2	6,910
100	20.9	7.2	159	15.4	.1	6,262
110	20.8	7.2	162	15.9	.2	5,335
II. ELEPHANT BUTTE RESERVOIR[2]						
S[3]	33.0	8.1	1,009	29.5	7.1	6,823
3	33.0	8.1	1,062	28.7	7.2	6,823
6	23.8	8.1	1,024	28.7	5.9	14,344
10	23.0	8.1	1,017	28.1	5.5	13,414
20	22.8	7.6	1,022	28.4	6.1	28,874
30	21.5	7.6	1,081	29.3	5.9	30,254
40	19.8	7.6	1,107	29.1	6.2	63,987
50	19.6	7.6	1,068	29.2	6.5	63,987
60	18.8	7.6	1,070	29.0	6.6	63,987
70	18.0	7.6	996	29.6	6.8	61,237
80	17.8	7.6	1,067	29.8	5.1	61,237
90	17.6	7.6	1,045	30.2	5.1	63,987
100	17.1	7.6	1,059	30.7	5.3	63,987
110	16.9	7.6	1,052	30.4	5.1	61,237
125	16.9	7.5	1,123	30.7	4.7	63,987
III. LAKE KEOKUK[4]						
S[3]	27.8	7.5	285	27.8	6.7	1,170
10	27.5	7.5	287	28.0	6.6	1,170
20	27.0	7.5	285	28.3	5.8	1,103
33	27.0	7.5	290	28.3	5.4	1,065

[1] Tennessee River near Florence, Ala., station 385, July 30, 1931, air temperature 31.3° C. A deep river lake showing midsummer stratification.

[2] Rio Grande River, near Hot Springs, N. Mex., station 829, June 15, 1935, air temperature 33.0° C. A deep lake showing partial stratification at time of these observations.

[3] Surface, i. e., top 12 inches of water.

[4] Mississippi River near Keokuk, Iowa, station 80, Sept. 22, 1932, air temperature 30.3° C. A medium to shallow river lake showing little or no stratification.

Table 5.—*Water temperatures of inland streams, exclusive of headwaters in mountainous regions, during warm season, June to September, inclusive, 1930–35*

River system	Number of stations	Number of cases	Percent of cases						Minimum degrees, Centigrade	Maximum degrees, Centigrade
			14.0°–17.9° C.	18.0°–21.9° C.	22.0°–25.9° C.	26.0°–29.9° C.	30.0°–33.9° C.	Above 34° C.		
Composite[1]	726	4,545	13	16	35	31	5	(2)	15.0	36.6
Entire Mississippi system	447	2,887	12	12	46	27	3	(2)	15.0	35.2
Coastal streams into Gulf of Mexico[3]	39	1,367	7	14	32	36	9	2	16.8	36.6

[1] Includes flowing streams at stations at which fish were found in Mississippi, Missouri, Tennessee, Ohio, coastal streams flowing into Atlantic Ocean and Gulf of Mexico, Rio Grande, Colorado, and Columbia system.

[2] Less than 1 percent.

[3] Exclusive of Mississippi system.

of fishes. Besides, portions of the bottom usually in the shallower parts of the stream provide nesting sites for certain species. However, as the stream bed may change from rock and coarse gravel to fine clay in the course of a few hundred yards, and as there is a wide variation in habitat preference of the many desirable organisms which inhabit stream bottoms, it is very difficult to define all of the conditions at the bottom of a stream which may or may not be suitable for bottom inhabitants and therefore affect the fish fauna; for over this already complicated association of rock, soil, debris, and living organisms pollution may spread a blanket of silt, sludges, pulps, and poisonous fluids.

Studies of the bottom faunae of polluted streams, particularly those carrying quantities of organic pollution, have shown that many of the bottom species of unpolluted streams are very sensitive to pollution conditions, so that as pollution progresses the normal bottom fauna changes giving way to certain more tolerant species of tubificid worms, chironomid midges, sphaeriid mollusks, and leeches. Consequently, various investigators (Marsson, 1911; Suter and Moore, 1922; Turner, 1927; Richardson, 1928; Wiebe, 1928), in connection with other data have correlated the presence of certain bottom forms with different degrees of stream pollution, and a few such species of bottom organisms have come to be used to some extent as indices of pollution. Richardson (1928) however, in discussing 49 bottom species definitely associated with pollutional or subpollutional conditions in the Illinois River points out the difficulties attending the use as indices of pollution, of even such species as the tubificid worm, Tubifex tubifex, and the midge, Chironomus plumosus, which are commonly regarded as characteristic of pollutional conditions bordering on septic. The problem of index species is greatly complicated by the fact that the number of individuals of any particular index species may vary from zero over a wide range in adjacent parts of any polluted stream as the result of conditions other than those produced by pollution.

In the present studies, bottom samples were taken throughout the field work as a part of the regular routine. Reviewing the results of the examinations of many hundreds of bottom samples, there is no doubt that bottom samples are a necessary part of any pollution study, and that the findings concerning the bottom organisms are very valuable when considered with the chemical and physical data. However, the writer agrees with the statement of Richardson (1928, p. 410), in connection with his Illinois River studies, that index species are of service in pollution studies only when used with the greatest caution, and when checking with other indicators.

With due regard to the limitation just discussed, three organisms -- namely, the water mold, Sphaerotilus natans, the rattail maggot, Eristalis sp., and the sludge fly, Psychoda sp. -- were often found helpful in delimiting the septic zone near the source of extensive organic pollution; and the tubificid worms, Tubifex sp. and Limnodrilus sp., and the larvae of the red midge, Chironomus sp. (particularly Chironomus plumosus), if their presence or absence were supported by the chemical and physical analyses and by the data concerning the biological complex as determined from repeated samplings, were frequently used in defining the extent of bad to moderate organic pollution. In the lesser degrees of organic pollution and in most cases of chemical pollution index species as such were of little value.

On the other hand, the abundance of the various species comprising the bottom fauna and particularly the species composition of the bottom fauna at various stations in the polluted area were usually of large value, regardless of the sort of pollution, when correlated with the physical and chemical data. Gross pollution of several kinds was quickly detected from bottom samples both by the physical and chemical conditions of the mud itself and by the paucity or absence of bottom fauna, often represented only by dead shells and other remains. Dredgings readily demonstrated such gross pollution by silt, sewage, pulps, tars and heavy oils, mine tailings, rock powders from quarry operations, and such organic wastes as starch effluents, sizings from cloth mills, and distillery slops.

Downstream from a region of gross pollution the biological analyses of the bottom faunae were often particularly significant when compared with the bottom faunae in similar situations in comparable unpolluted waters (if possible of the same stream) in showing the selective effects of some pollutants, and the downstream extent of pollution.

Many chemical wastes have high specific gravity and consequently tend to follow the stream bottom for some distance before complete mixing is accomplished by stream current action. Often downstream dredgings showed complete absence of bottom life of any type for some distance below the outlets of flumes carrying such chemical wastes, and the limits of the zones in which the bottom faunae had been killed out yielded valuable information concerning the points at which the effluent had been diluted to a nontoxic level.

ACTION OF POLLUTANTS ON FISHES

Regardless of the effects on the environment, many pollutants affect fishes directly by some specific action on the living organism itself. These pollutants can be grouped, therefore, according to the locus of injury and method of action of the various active substances which the effluents contain (Ellis, 1936b).

INJURIES TO GILLS AND EXTERNAL STRUCTURES

The higher concentrations of almost all effluents and all lethal concentrations of some types of pollutants kill by their actions on the gills of the fish before little, if any, of the material causing the death of the fish passes beyond the gills. Such substances kill, therefore, by a combination of chemical and physical injuries rather than by true toxic action. The salts of several heavy metals, some acids, and some special chemicals as trinitrophenol combine readily with the mucus secreted by the fish's skin, mouth, and gills, forming insoluble compounds.

If the dilution of the pollutant of this type be great enough, or if the supply of the pollutant be limited so that it acts on the fish only a short time, the secretion of additional mucus may wash away the precipitated compound as fast as it is formed, or may carry away the larger masses of the precipitate before serious damage to the fish results. If, however, the concentration and exposure time are large enough (very small quantities of several heavy metal salts suffice, v.i.), the precipitated insoluble compound covers the body, the lining of the mouth, and the gills. (See Fig. 21.) Disastrous results follow the covering of the gills with these precipitates, and death supervenes very rapidly from the respiratory failure. In our experiments with salts of heavy metals and various other compounds producing death by precipitation of insoluble matter on the gills, the precipitate was found to cause death as the result of a combination of three conditions which individually or collectively disturbed the proper functioning of the respiratory and circulatory mechanisms. In the weaker concentrations of such effluents and harmful substances, the precipitate coated the gill filaments and filled the filament interspaces so that the water pumped through the mouth and onto the gills for the aeration of the blood could not reach the cells of the gill filaments. Consequently, the aeration of the blood with its accompanying gas exchanges was prevented, and sooner or later death followed from a combination of anoxemia and carbon-dioxide retention.

If still larger masses of flocculent precipitate were formed, as was the case when the stronger effluents were tested, the interlamellar spaces were clogged and movement of gill filaments became impossible. This condition affected the circulation of the blood in the gills, in that stasis of the blood in the gill capillaries usually follows cessation of movement of gill filaments in a short time. Kolff (1908) in her studies of fish hearts found that the pulsation of the heart is only one of the forces necessary to drive the blood through the gill capillaries, and that the movements of the gills are important in maintaining the gill capillary circulation.

If the scales be removed from the midventral portion of the precardial region of a pithed goldfish and a tiny steel needle (the minuten nadeln on which entomologists mount very small insects) be forced through the body wall and into the pericardial cavity, when proper adjustment is made, this needle, if examined under a binocular microscope, can be seen to swing with each heart beat. Using this procedure the rate of the uninjured and unexposed heart in situ can be recorded for hours. Such fish, when the gills were perfused via the mouth with water containing salts of heavy metals or other substances producing these heavy mucoid precipitates, continued their normal respiratory movements and heart action until the precipitate clogged the gills and mechanically prevented the movement of the gill filaments. At that time an abrupt change in the activities of the heart was usually noted, the heart action dropping to about one-half its former rate. Heart block also often developed at this time. When these changes in heart rate and heart action were noted, the gill capillaries were always found to be gorged with blood on the efferent or cardiac side and showed practically complete to complete stasis of the blood in the capillaries. This same condition of blood stasis in the gill capillaries was noted in other fish in which anoxemia was produced by exposure to water containing little or no dissolved oxygen shortly after cessation of respiratory movements, so that the importance of the respiratory movements for the maintenance of capillary circulation in the gills was well established. After its rate had dropped to approximately one-half normal, the heart continued to beat for hours (in some cases for over 24 hours), although the circulation through the gills was completely blocked. In fishes which had ceased to make movements of any sort, either respiratory or general, after prolonged exposure to water low in dissolved oxygen, the hearts were found to be beating even when these fishes were opened under oil to exclude even momentary aeration of the pericardial fluid.

The various substances producing these heavy precipitates, as well as many other pollutants (acetic acid, volatile extracts of crude oil, and others) which did not form precipitates with mucous secretions on the gills, also produce death by asphyxia through direct damage to the gill filament cells, which in turn cause stasis of the blood in the gill capillaries. Death from anoxemia and circulatory failure in the gills, therefore, was regularly found in fishes dying either in waters containing low oxygen or in the stronger hypertonic solutions of various salts and other chemicals, regardless of the formation of any sort of mucoid precipitate.

In connection with gill damage, it must be pointed out that the weaker concentrations of various pollutants which do not damage the gills rapidly enough to cause speedy respiratory and circulatory failures must also be considered as pollution hazards if these solutions be even slowly toxic to the cells of the gill, for Smith (1929) has demonstrated that the gills of fresh-water fishes (carp and goldfish) are important excretory structures, removing from 6 to 10 times as much nitrogenous wastes as the kidneys. Keys and Willmer (1932) have found special cells associated with chloride secretion in the gills of various fishes, including fresh-water species. Damage to the gills from pollutant substances in the water can result, therefore, in the impairment of other functions, particularly those of salt balance and excretion in addition to respiration and circulation as already described, without actual toxic action on the internal vital organs of the fish.

POLLUTANTS ENTERING THE BODY OF THE FISH AND EXERTING TRUE TOXIC ACTION

The gills, the lining of the mouth, and the skin are the main portals of entry through which toxic substances can be absorbed into the body of the fish. However, Bond (1933) concludes that the bony fishes and many fresh-water invertebrates have little or no permeability to a variety of substances. The writer has been able to confirm this relative nonpermeability of the external structures of various fresh-water fishes, particularly the gills, for a number of substances. The active agents in various stream pollutants, therefore, may be still further subdivided into those which can enter the body of the fish through external structures as various volatile compounds from crude oil, dilute chlorine, ether, chloroform, methyl mercaptan, and formaldehyde, and those which do not

BULLETIN U. S. B. F., 1937. (Bull. No. 22)

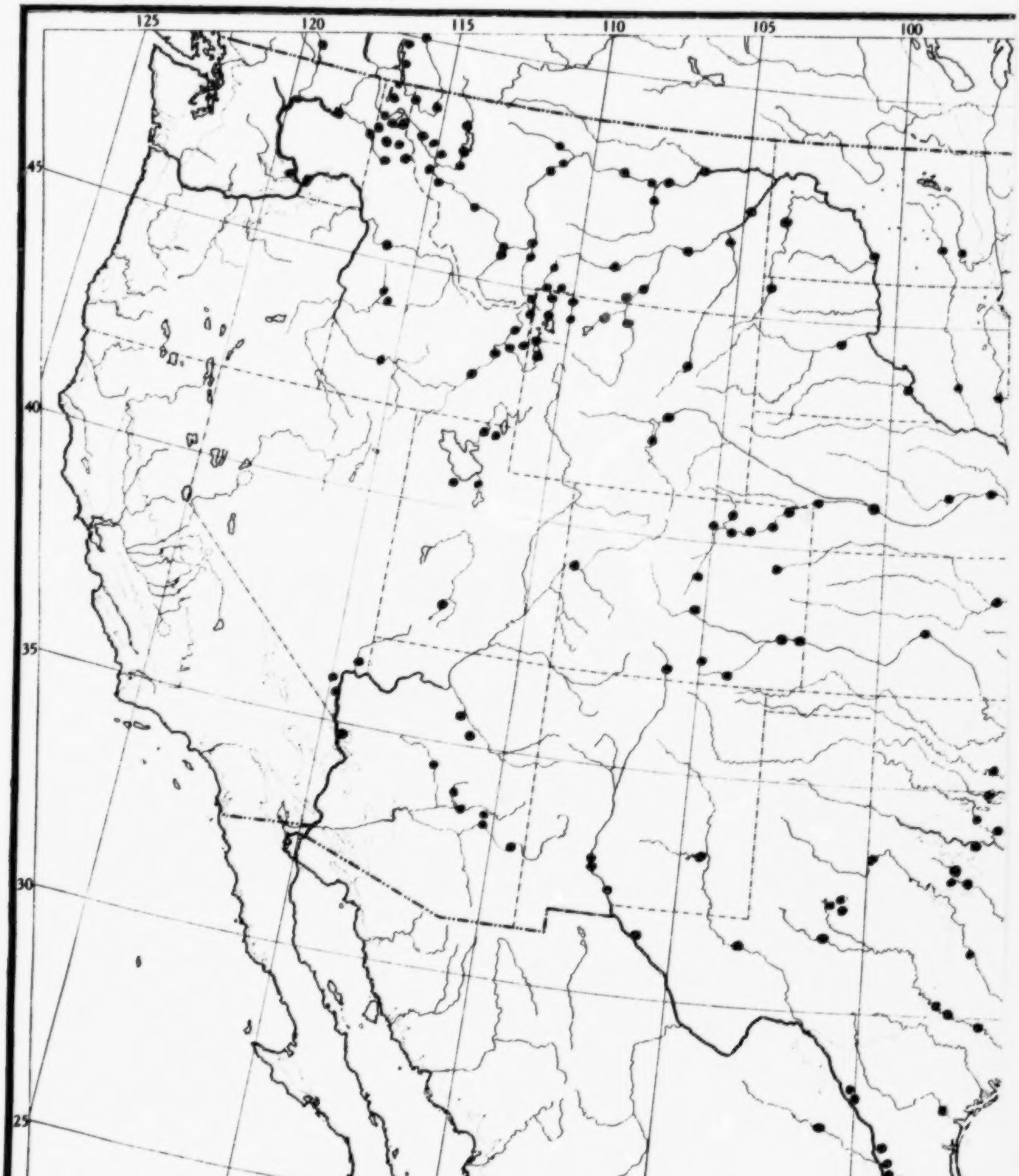
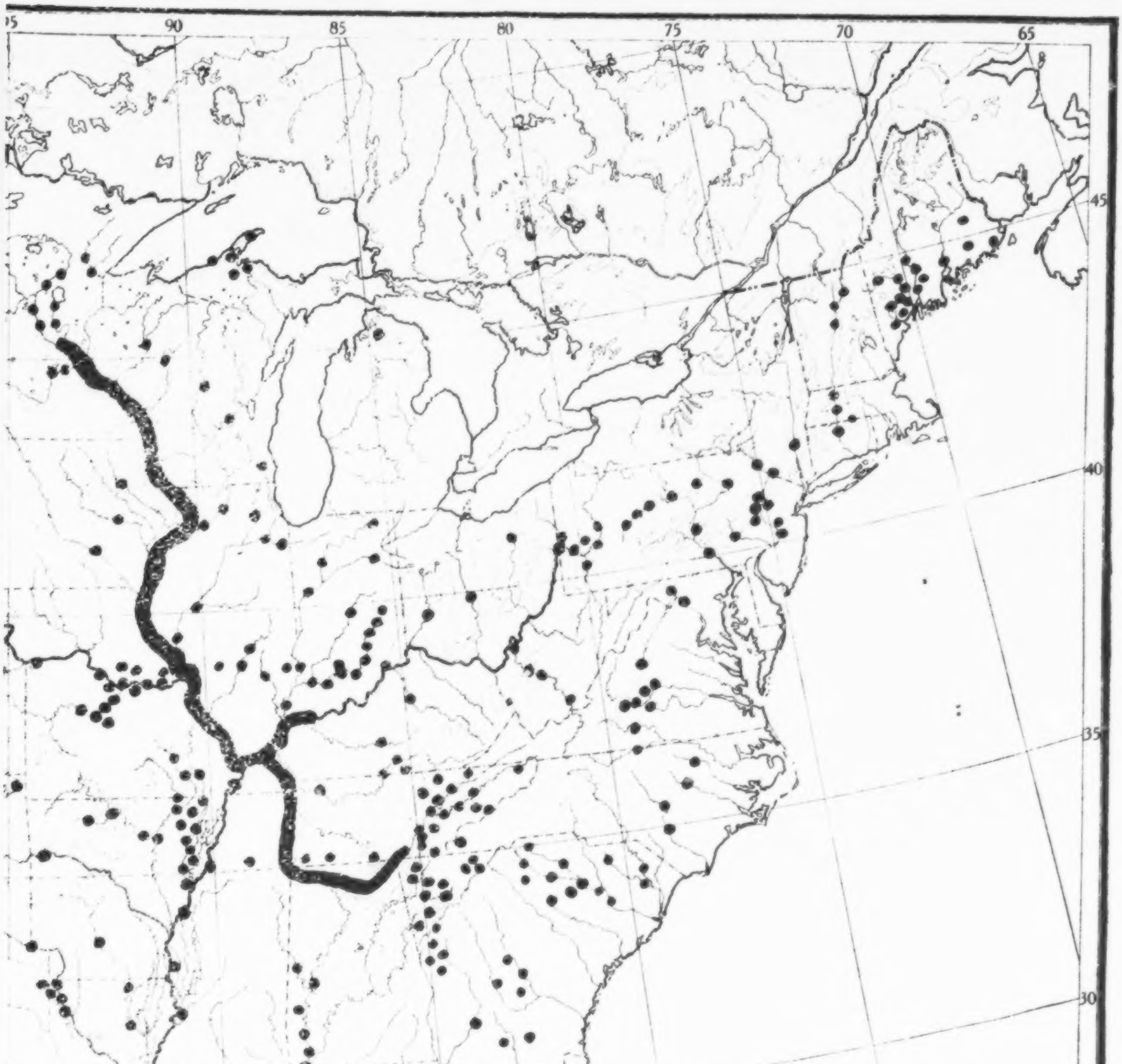


Figure 20 - Map showing the rivers surveyed by U.S. Quarterboat 348 (heavy black lines) and the major base poin



lack spots) from which field trucks operated in the accumulation of field data presented in the foregoing sections.

enter or at least enter very slowly through the external structures as certain arsenic compounds.

The main internal channel for the absorption of injurious and toxic substances is the gastrointestinal tract. The writer, using colloidal dye methods, has found that many fresh-water fishes refuse to swallow water containing various types of effluents for sometime after being placed in such water, for as has been pointed out by Smith (1930) most of the water required for the formation of urine in the fresh-water fish is probably taken in through the lining of the mouth. However, after varying intervals, usually 48 hours or more, fresh-water fish swallow some of the surrounding water even if not fed, suggesting that the water needs of the body are not entirely supplied through absorption by the external surface or the lining of the mouth. The swallowing of polluted water allows any of the injurious or toxic substances which this water may contain direct access to the mucosa of the gastrointestinal tract. Harmful substances may, therefore, either damage the lining of the intestinal tract or may be absorbed from the gastrointestinal tract, and in this way gain access to the internal vital organs. Consequently, another group of pollutants can be designated as those acting on the fish after ingestion into the gastrointestinal tract. It is through this portal that many cumulative poisons enter the body of the fish during long-time exposures to concentrations of pollutants which may at the time seem harmless. Arsenic may be cited as an example of a substance which can be accumulated by the fish via the gastrointestinal tract after long exposure to low concentrations.

To summarize, on the basis of locus of injury to the fish, stream pollutants may be classified as --

(1) Those substances injurious to the gills and other external structures of the fish without any marked absorption beyond the gills. Death of the fish exposed to these pollutants results from anoxemia, through respiratory and circulatory failure, and through interference with the excretory functions of the gills.

(2) Those substances killing the fish by specific toxic action after absorption through the gills, the lining of the mouth, and other external structures.

(3) Those substances killing the fish by specific toxic action after absorption from the gastrointestinal tract, which region they have reached in water swallowed by the fish.

LETHALITY OF SPECIFIC SUBSTANCES OCCURRING IN STREAM POLLUTANTS

GENERAL CONSIDERATIONS

In this section, data from the literature and from experimental assays made during the present studies are presented covering the lethality of various substances which are the active harmful agents in different types of stream pollutants. These biochemical and biophysical findings in general may be applied to pollution conditions ranging from acute to mild; although in the evaluation of all individual cases of pollution, caution must be exercised, as biological data of any sort are rarely suitable for rigid application. One particular component of a given waste may constitute the major pollution hazard of that waste; in fact, the dangerous qualities of many wastes center largely around one substance in each effluent rather than the effluent mixture as a whole. In such cases, lethality tables are particularly helpful, but the synergistic and antagonistic actions of other substances in the stream water as well as in the waste itself must be borne in mind always. For example, the lethality of copper sulphate for fishes under certain conditions is decreased by the presence of calcium chloride and increased by sulphuric acid, and both of these compounds occur free or combined in certain industrial wastes. Again the toxic actions of small amounts of chlorine and of ammonia on aquatic life singly are different from the actions of the same quantities of these substances when both are present, since together they may form either ammonium chloride or chloramine, depending upon conditions; and the toxicity of the resultant mixture may vary accordingly.

From the data presented in the following tables, however, the harmless, injurious, and lethal ranges of various substances may be computed readily with reasonable accuracy for particular pollution cases if due regard be given to the chemical and physical conditions of the water of the stream involved. Tests should be made, of course, to detect the presence of unusual and unexpected substances in the local complex which might alter the action of the pollutant.

In addition to the appraisal of the pollutant on the basis of the lethal action of its major component or components, when the pollution is acute, mild, or intermittent, under which headings a large percent of the pollution cases may be classified, the possibility of cumulative effects must be considered when determining remedial measures or dilutions. Cumulative effects from very low concentrations of some substances which seem harmless to fish life over a period of 10 to 14 days present difficult problems. Some of these are discussed in subsequent portions of this section.

TEST ANIMALS

In the course of the work on the lethality of stream pollutants 30 species of fresh-water animals (see Table 6), including 17 species of fish, 6 species of crustaceans, and 7 species of fresh-water mussels, have been used in one connection or another as test animals.

From these studies and from the voluminous literature of bioassay methods and physiological and pharmacological experiments in which these and many other forms of aquatic life have been used as bioreagents, two animals -- the common goldfish, Carassius auratus, and the entomostracan, Daphnia magna -- were selected for the standard tests. This procedure, i.e., the use of standard test animals, is recognized in other fields of biological investigation in which the frog, the rat, and the guinea pig have established positions as assay animals, and makes possible direct comparisons of data on the actions of a variety of substances over a wide range of conditions. No single test animal combines all of the desirable qualities and

TABLE 6.—*Species of test animals*

Species	Common name	Species	Common name
FISH		AMPHIPODS	
1. *Perca flavescens*	Yellow perch.	18. *Gammarus fasciatus*	Gammarid.
2. *Micropterus salmoides*	Largemouth black bass.	19. *Eucrangonyx gracilis*	Do.
3. *Lepomis incisor*	Blue gill.		
4. *Lepomis humilis*	Orange-spotted sunfish.	ENTOMOSTRACANS	
5. *Ameiurus nebulosus*	Common bullhead.	20. *Daphnia magna*	Daphnia.
6. *Ictalurus punctatus*	Channel cat.	21. *Bosmina sp*	Cladoceran.
7. *Agosia nubila*	Long-nosed dace.	22. *Cypris sp*	Ostracod.
8. *Leuciscus balteatus*	Dace minnow.	23. *Cyclops* sp	Copepod.
9. *Notemigonus chrysoleucas*	Golden shiner.		
10. *Notropis delicious*	Straw-colored minnow.	FRESH-WATER MUSSELS	
11. *Hybognathus nuchalis*	Silvery minnow.	24. *Tritogonia verrucosa*	Buckhorn.
12. *Pimephales promelas*	Fathead minnow.	25. *Megalonaias gigantae*	Washboard.
13. *Cyprinus carpio*	German carp.	26. *Quadrula trapezoides*	Small washboard.
14. *Carassius auratus*	Common goldfish.	27. *Fusconaia undata*	Pigtoe.
15. *Salmo irideus*	Rainbow trout.	28. *Actinonaias carinata*	River mucket.
16. *Salmo clarkii*	Cut-throat trout.	29. *Lampsilis anodontoides*	Yellow sand-shell
17. *Lepisosteus platostomus*	Short-nosed gar.	30. *Lampsilis siliquoidea*	Fat mucket.

in making a choice both availability and physiological suitability for the problem in hand must be considered. As the variety of aquatic animals which may be affected by stream pollution is large, a lethality range for each pollutant and effluent must be established, as the specific sensitivities of various aquatic species are not the same, although they may live in the same aquatic complex. However, if the pollutant destroys any one of the major species in the complex, the entire fauna in that part of the stream may suffer as a result. The selection of goldfish and daphnia gave a combination by which the maximal and minimal limits of toxicity of any given pollutant for different stream complexes could be ascertained rapidly and with reasonable accuracy.

GOLDFISH

Goldfish of any size up to 6 inches are easily obtainable in quantity from commercial goldfish farms, are relatively inexpensive, and stand shipment well. The practical considerations, although important in the selection of a test animal, are of course secondary to the physiological characteristics of the goldfish which make it particularly suitable for the bioassays of stream pollutants. The goldfish is tolerant of confinement and can be kept in laboratory containers and aquaria for months, making both short-time and long-time tests, uncomplicated by excitement and the attendant endocrine distrubances, possible under controlled conditions. The amount of oxygen consumed by the goldfish is not large per unit of weight, and this fish does not succumb to low oxygen as quickly as many other fresh-water fishes. In fact, the uninjured goldfish if permitted can obtain sufficient oxygen to maintain itself for some time in water very low in or even devoid of dissolved oxygen merely by skimming and breaking the surface film of the water. Because of these facts, the toxic action of many substances which would be volatilized or oxidized by the constant aeration of water required to maintain various other species of fish can be ascertained, using goldfish as reagents; and the specific parts played by the oxygen demand, the external action, and the internal effects of the pollutant on the fish determined separately. On the other hand, if the combination of anoxemia and toxic action of the pollutant are desired, goldfish can be placed in closed containers, or merely prevented from reaching the surface layer of the water by submerged strips of glass which do not interfere with either the movement of the water or with its surface reaeration.

By comparative tests the relative resistance of the goldfish to poisons, injury, and anoxemia was found to be equal to or greater than that of bass, perch or catfish, and definitely greater than the resistance of trout or most fresh-water minnows. The minimal lethal concentration of any effluent or pollutant for goldfish established, therefore, approximately the maximal concentration of that pollutant which ought not to be exceeded if any fresh-water fish were to survive in waters polluted with such effluent.

An added advantage in the use of the goldfish as a test animal is the already large literature on this fish in both physiological and pharmacological fields, in which the usefulness of this animal as a bioassay reagent is well established (Munch, 1931; Powers, 1917).

The stock goldfish used for these tests were obtained in lots as needed from a commercial goldfish farm, and were held in flowing water in large hatchery tanks at a temperature ranging from 17° to 21° C. All goldfish were of the standard carp-form variety, i.e., the various ornamental varieties were excluded. The fish were fed regularly every third day a ration of prepared shrimp meal, and Myriophyllum growing in the tank was available at all times. For routine tests 3-inch goldfish (60 to 90 millimeters in length), weighing between 3 and 5 grams, were selected as standard. Larger sizes were used for special tests as required.

DAPHNIA MAGNA

The second test animal, Daphnia magna, which is found in the quiet backwaters of various river systems (Surber, 1936), and which is raised extensively as food for young fish, was chosen because of its high sensitivity to most stream pollutants. Comparative tests

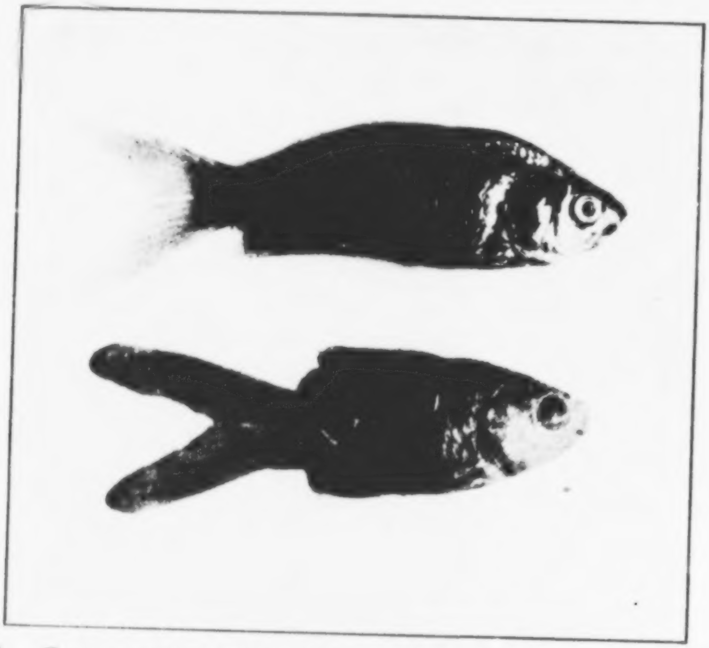


Figure 21 - Top, normal goldfish; bottom, goldfish killed in copper sulphate. Note thick deposit of opaque copper-mucoid precipitate covering body, fins, and eyes. This type of precipitate is produced by many wastes containing compounds of heavy metals.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 22 - Constant-temperature apparatus in which Daphnia magna were used as test reagents for toxicity of various stream pollutants. Assistant at the right is operating a concentrator preparatory to counting Daphnia.

showed this animal to be more sensitive even than trout, so that the minimal lethal concentration for daphnia of a given pollutant gave approximately the maximal dilution from which damaging effects on fresh-water fishes and many other fresh-water animals could be expected (exclusive of certain cumulative effects). Consequently, between the minimal lethal concentration for daphnia on the one hand and the minimal lethal concentration for goldfish on the other, the gamut of lethal concentrations of any pollutant or effluent under conditions comparable to those of particular pollution cases could be defined rather definitely.

Daphnia proved to be an excellent test animal with which to obtain a quick orientation concerning the relative toxicity of effluents of unknown lethality, since this animal is quite free from the protective mucous secretion which safeguards fish for a time against many pollutants.

Daphnia were also valuable in studying the cumulative effects of some pollutants, as under favorable controlled conditions a new parthenogenetic generation could be expected every 5 to 8 days.

Like that of the goldfish, the scientific literature on daphnia is extensive, and the general physiology and reactions of this animal are well known. The use of daphnia as a bioreagent is also well established (Munch, 1931; Adams, 1927; Billiard, 1925).

The daphnia used in these tests were raised from original stock secured in Texas, pedigreed strains being established and lines of parthenogenetic clones secured for the assay work. The daphnia colony is maintained in a series of large glass jars, each containing four liters of water; and the animals fed bacterial nutrient material prepared from cottonseed meal as described by Chipman (1934).

All assay tests were made in a constant temperature cabinet (see Fig. 22) at 25° C. One hundred fifty daphnia of the same age (usually 4 days old) from the same strain were placed in each jar containing 3 liters of the solution to be tested, i.e., in a known dilution of the pollutant, using water from the same source as that in which the daphnia were living as the diluent. No abrupt changes in temperature were permitted, and the solution was well aerated before the daphnia were placed in it. Every 48 or 72 hours, depending upon the type of test, the animals in each jar were concentrated in a pyrex glass trap devised for this work, counted, and returned to the same or fresh solution as desired. When young appeared they were removed and carried in separate jars containing the same concentration of the pollutant as that of the original jar. In this way the mortality of each succeeding generation as well as the rate of reproduction could be followed.

WATER TYPES

When determining the specific lethality of any given pollutant for a particular case, water from the stream into which the effluent was being poured, taken just upstream from the point at which the pollutant entered the stream and filtered through bolting cloth to remove large masses of suspensoids and the macroplankton was used as the diluent water. In this way the pollutant was mixed with the same water as in the actual case of pollution under consideration and the same chemical interreactions were possible. When it was not feasible to use stream water at the site of the pollution, unpolluted water from a comparable stream was diluted or fortified until the salt content, the conductivity, the carbonates, and the pH were essentially those of the stream water in question. This was made possible by the field and laboratory analyses of the waters of the stream. This method of compounding a synthetic stream water, although laborious at times, gave very satisfactory comparative results. Control tests were also made in each investigation using glass-distilled water as the diluent for certain critical concentrations.

In making the assays of pollutants, regardless of the diluent water, no material was removed after making the dilution; i.e., even if precipitates or colloidal suspensions were formed on the addition of the pollutant to the diluent water, the fishes or daphnia were exposed to the action of the entire mixture. Many substances which are precipitated in stream and river water are nevertheless slightly soluble in water, particularly if small quantities of carbon dioxide and other compounds be present, so that portions of the precipitate might continue to redissolve for hours or days, and insoluble substances even though precipitated to the bottom of the stream constitute supplies of various ions which may be drawn into the water as others are removed. Again, some of these precipitated materials are dissolved or chemically changed by the action of various substances in or on the body of the fish or daphnia, or by the excretory products of these animals, so that particles of precipitated material lodging temporarily on the gills might be dissolved by the carbon dioxide there, or other particles swallowed by the fish might be acted upon by the digestive fluids of the animal. In view of these complicating factors, the tests made in stream waters are much more valuable in obtaining the actual hazards to aquatic life than those made in distilled water.

In the basic data tests (see Tables 7, 8, 9, 10, 11, 12, and 13) four types of water were used as diluents, namely, glass-distilled water for the chemical controls in which the specific and uncomplicated action of the substance was studied, and soft, medium, and hard water (following the classification of Birge and Juday, 1911), i.e., with fixed carbonates less than 5 cubic centimeters per liter, 5 to 22 cubic centimeters per liter and over 22 cubic centimeters per liter, respectively, for the general lethality tests. Ranges of lethality were obtained in this way which are applicable within the limitations previously discussed to most stream waters of the United States, since the hardness of these natural stream waters is dependent almost entirely on the calcium and magnesium carbonates which exists in different degrees of concentrations in the interior waters.

The soft and hard natural waters were obtained at Columbia, Mo.; the hard water representing a typical limestone drainage, and the soft water a typical clay drainage. The medium water was obtained from the upper Mississippi River. In the tables the pH and *specific conductance are given both for the original* water and for the mixture as modified by the pollutant, so that the fluctuations in the diluent water itself and in the mixture as the result of the action of the pollutant on the salt and buffer balances may be followed in each case.

SPECIFIC LETHALITY TABLES

Osmotic Pressure and Sodium Chloride

Many effluents carry soluble substances exerting considerable osmotic pressure, and because of that property are capable when sufficiently concentrated of withdrawing water from the gills of fishes and from other delicate external organs of various aquatic organisms, with the attendant damage to the living cells. High concentrations of many sorts of pollutants present this danger quite independently of any toxic, chemical, or corrosive action they may have on aquatic life. The toxic and chemical actions of various salts in stream pollutants may be in part offset by the presence of other substances; but the osmotic action of all of the components of the mixture may be lethal regardless of their mutual antagonisms or specific toxicities, consequently the osmotic pressures of concentrated effluents must be regarded as potential hazards until these substances are sufficiently diluted to be within the limits of osmotic pressure tolerated by fresh-water fishes.

Garrey (1916) finds, using the straw-colored minnow, Notropis blennius, as a reagent, that fresh-water fishes tolerate an osmotic pressure of the external medium equal to that of their own blood if the various salts and substances in the water are balanced against each other so as to exclude the specific toxic effects. As the blood of fresh-water fishes contains approximately 0.7-percent sodium chloride, together with small quantities of calcium and potassium salts, the limit of osmotic pressure tolerated in the external medium by fresh-water fishes is therefore near 6 atmospheres, or the approximate equivalent of 7,000 p.p.m. of sodium chloride (common salt) in the surrounding water.

In Table 7 data are presented showing the effect of common salt in medium water (Mississippi River water) on test goldfish. This water, of course, contained small quantities of calcium and magnesium salts, which served to some extent to offset the specific toxic action which sodium chloride has in distilled water. (See sodium chloride, p. 177.) These river-water tests confirm the work of Garrey (1916), as sodium chloride killed quickly in concentrations greater than 10,000 p.p.m., and was not lethal in concentrations of 5,000 p.p.m. or less. The survivals in a concentration of 10,000 p.p.m. sodium chloride in river water ranged from 4 to 7 days, and the predicted maximal nonlethal concentration of sodium chloride on the basis of osmotic pressure alone in terms of the osmotic pressure of fish blood would be approximately 7,000 p.p.m.

In practical pollution work, therefore, any effluent as long as its osmotic pressure is greater than 6 atmospheres may be expected to be lethal to fresh-water fishes regardless of any specific toxic properties. Below this osmotic pressure the toxic, chemical, and corrosive characteristics of wastes will be the major determining factors in its lethality. Brine wastes, however, often present pollution hazards largely because of their osmotic pressures.

ACIDS

High acidity is a characteristic of a large number of wastes, so that the action of uncombined acid is one of the major problems of stream pollution. In determining the lethality of acid wastes, both the actual acidity -- that is, the pH or hydrogen-ion concentration of the polluted water carrying acid effluent -- and the specific acid involved must be considered, as acid wastes do not kill merely because of a particular degree of acidity.

In Table 8 a summary of an extensive series of experiments on the lethality of 11 acids found in stream pollutants is presented. It is obvious, first of all, from this table that it is futile to attempt to designate a given p.p.m. value as marking the lethal point for any particular acid as the buffer substances and dissolved salts of the water into which acid waste is poured will determine the amount of free acid or hydrogen-ions available. The actual conditions, therefore, presented to aquatic life following the addition of a given number of p.p.m. of a particular acid to soft water will be quite different from those presented by the same amount of that acid when added to hard water, with of course all sorts of intermediate possibilities between these two extremes. For example, 134 p.p.m. of sulphuric acid killed goldfish in from 6 to 90 hours when added to soft water, but were not lethal when added to hard water.

TABLE 7.—*Survival of goldfish in solutions of sodium chloride: diluent, filtered Mississippi River water*[1]

Concentration ration by weight	Parts per million	pH		Specific conductivity mho $\times 10^{-6}$ at 25° C.		Survival time[2]
		Water	Solution	Water	Solution	
1:20	50,000	7.8	7.7	236	62,669	30 to 40 minutes.
1:50	20,000	7.8	7.7	235	31,336	1 to 2 hours.
1:67	14,925	7.8	7.7	235	25,254	10 to 12 hours.
1:80	12,500	7.8	7.7	235	25,200	24 to 36 hours.
1:100	10,000	7.8	7.7	235	18,772	4 to 10 days.
1:200	5,000	7.8	7.7	235	4,156	∞
1:1,000	1,000	7.8	7.7	235	2,328	∞
1:10,000	100	7.8	7.7	235	455	∞
1:100,000	10	7.8	7.7	235	245	∞

[1] Condition of experiments as described in table 8.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 25 days without any apparent injury to fish.

TABLE 8.—*Survivals of 700 goldfish in various concentrations of 11 acids found in industrial wastes* [1]

Substances	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho$\times 10^{-6}$ at 25° C.		Survival time [1]	Constituent of—
				Water	Solution	Water	Solution		
Acetic acid	1:70	14,286	Hard	8.0	3.0	641	936	26 to 33 minutes	Beet-sugar wastes.
Do	1:201	4,975	do	8.0	3.5	641	749	30 to 36 minutes	
Do	1:631	1,585	do	8.0	4.0	641	673	50 to 60 minutes	
Do	1:1,000	1,000	do	7.8	4.5	645	693	50 minutes to 1 hour 30 minutes.	
Do	1:1,005	995	do	8.0	4.5	641	652	2 hours to 5 hours 50 minutes.	
Do	1:1,077	929	do	7.9	4.6	641	662	1 to 6 hours	
Do	1:2,365	423	do	8.0	5.0	641	676	4 hours 45 minutes to 20 hours.	
Do	1:2,871	348	do	8.0	5.5	641	675	20 hours to ∞	
Do	[2] 1:10,000	100	do	7.8	6.8	645	675	48 hours to ∞	
Do	[2] 1:100,000	10	do	7.8	7.3	645	688	∞	
Do	[2] 1:1,000,000	1	do	7.8	7.6	645	680	∞	
Benzoic acid	1:1,000	1,000	do	7.8	4.8	666	593	29 to 50 minutes	Coal-tar wastes.
Do	1:5,000	200	do	7.8	5.9	666	598	7 hours 16 minutes to ∞	
Chromic acid	1:1,000	1,000	Very soft	6.2	1.4	<50	3,680	15 to 23 minutes	Tannery wastes.
Do	1:1,250	800	Hard	7.8	5.4	647	844	3 hours 21 minutes	
Do	1:2,500	400	do	7.8	5.9	647	724	15 hours 18 minutes to 28 hours 24 minutes.	
Do	1:5,000	200	do	7.8	6.4	647	716	60 hours 24 minutes to 84 hours.	
Do	1:10,000	100	do	7.8	7.3	647	689	∞	
Do	1:10,000	100	Very soft	6.2	4.0	<50	469	30 to 35 minutes	
Citric acid	1:400	2,500	Hard	8.0	3.0	641	841	55 to 86 minutes	Citrus-fruit products wastes
Do	1:698	1,433	do	8.0	3.5	641	654	3 hours to 3 hours 30 minutes.	
Do	1:1,119	894	do	8.0	4.0	641	542	4 to 28 hours	
Do	1:1,600	625	do	8.0	4.5	641	494	∞	

tartaric acids; pH 5.5, acetic acid; pH 5.8, oxalic acid; pH 5.9, benzoic acid; pH 7.3, chromic acid; and pH 7.8, tannic acid. To this comparison, it must be added, as previously pointed out in the section on pH of natural water, that Brown and Jewell (1926) found fish living in a bog lake where the acidity of the water was pH 4.5 due to the action of carbon dioxide and organic acids leached from the surrounding bog vegetation. It is evident from all of the data in Table 8 that no matter what the diluent water be, as far as hydrogen-ion concentration alone is concerned, the acidity near the magnitude of pH 4.0, regardless of the acid or acid-salt combinations producing this acidity, will be lethal for fresh-water fishes if that concentration of acidity be maintained. All acid concentrations more acid than pH 4.0 were lethal. In concentrations less acid than pH 4.0 the penetrative properties and the lethality of the kation of the acid must also be considered, for it may be seen that at any given pH the relative lethality varies with the particular acid involved.

On the basis of p.p.m. of acid present, disregarding the pH factor, the acids may be arranged in order of lethality as follows: Tannic acid, 10 p.p.m.; chromic acid, 100 p.p.m.; sulphuric acid, 130 p.p.m.; hydrochloric, 159 p.p.m.; benzoic, nitric, oxalic, and tartaric, each 200 p.p.m.; acetic acid, 348 p.p.m.; lactic, 430 p.p.m.; and citric, 625 p.p.m., in the particular hard water tested. The exact p.p.m. values are not so significant, for they will vary to some extent with the hardness of the diluent water; but the group shows the general order of magnitude of the relative lethality for these common pollutant acids.

Fishes are killed by acid wastes first through the precipitation and coagulation of the mucus on the gills and by the coagulation of the gill membranes themselves. If this coagulation of gills and gill secretions does not take place, the death of the fish is attributable to the lethal action of the kation of the acid. Combinations of these two actions are not uncommon, i.e., both precipitation and specific toxic action may contribute to the death of the fish. The precipitation of the mucus and of the proteins within the gill membrane cells themselves progresses rapidly when the relative acidity of the mixture is more acid than pH 4.5 because of the acidity itself, but this precipitation may be enhanced by the action of the kation of the acid. Tannic, chromic, and nitric acids all have marked affinity for living protoplasm, forming insoluble compounds with certain protein constituents of living tissue very promptly; in fact, all three of these acids are used as histological fixing agents to kill protoplasm. In the previous comparison on the basis of pH value, the minimal lethal concentration of these acids shows that tannic, chromic, and nitric killed goldfish in solutions less acid than the lethal solutions of sulphuric, hydrochloric, citric, and lactic acids. Acetic acid has high penetrative properties and causes a swelling of tissues which is very destructive to the living cells, so that although this acid does not coagulate the gill membrane and gill mucus like hydrochloric acid, it does disrupt the cells of gill membrane with

TABLE 8.—*Survivals of 700 goldfish in various concentrations of 11 acids found in industrial wastes* [1]—Continued

Substances	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho×10⁻⁶ at 25° C.		Survival time [2]	Constituent of—
				Water	Solution	Water	Solution		
Hydrochloric acid	[3] 1:1,000	1,000	do	7.8	1.8	680	3,481	10 to 15 minutes	Chemical wastes.
Do	1:5,100	196	do	8.0	3.0	641	1,221	1 hour 15 minutes to 1 hour 30 minutes.	
Do	1:5,614	178	do	8.0	3.5	641	930	1 hour 23 minutes to 2 hours 15 minutes.	
Do	1:6,011	166	do	8.0	4.0	641	912	4 hours 28 minutes to 6 hours 32 minutes.	
Do	1:6,299	159	do	8.0	4.5	641	853	∞	
Do	[3] 1:10,000	100	do	7.8	6.9	645	654	∞	
Do	[3] 1:100,000	10	do	7.8	7.5	645	650	∞	
Do	[3] 1:1,000,000	1	do	7.8	7.7	645	648	∞	
Lactic acid	1:326	3,068	do	8.0	3.0	641	1,100	40 to 50 minutes	Dairy industries wastes.
Do	1:706	1,416	do	8.0	3.5	641	765	1 hour 12 minutes to 1 hour 45 minutes.	
Do	1:1,530	654	do	8.0	4.0	641	600	6 to 43 hours.	
Do	1:2,324	430	do	8.0	4.6	641	566	∞	
Nitric acid	1:1,333	750	do	7.8	3.4	666	1,625	30 to 50 minutes	Chemical wastes.
Do	1:5,000	200	do	7.8	4.9	666	818	∞	
Do	1:10,000	100	do	7.9	7.9	652	698	∞	
Oxalic acid	1:1,000	1,000	do	7.8	2.6	666	2,620	25 to 30 minutes	Dye, tanning and bleaching wastes.
Do	1:5,000	200	do	7.9	5.3	652	405	∞	
Do	1:10,000	100	do	7.9	6.8	652	524	∞	
Do	1:17,000	59	Very soft	6.2	4.5	<50	146	∞	
Do	1:40,000	25	do	6.2	5.8	<50	119	∞	
Do	1:100,000	10	Hard	7.9	7.6	652	662	∞	
Sulphuric acid	[3] 1:1,000	1,000	do	7.8	2.2	645	1,381	30 to 45 minutes	Coal- and iron-mine waters.
Do	1:5,922	169	do	7.8	3.0	640	1,203	50 minutes to 1 hour 10 minutes.	
Do	1:7,000	143	Soft	6.4	3.5	583	790	2 hours 30 minutes to 5 hours 17 minutes.	
Do	1:7,000	143	Hard	7.8	3.5	640	1,170	2 hours to 2 hours 20 minutes.	
Do	1:7,250	138	Soft	6.4	3.9	583	753	5 to 6 hours.	
Do	1:7,250	138	Hard	7.8	4.0	640	868	4 hours to ∞	
Do	1:7,450	134	Soft	6.4	4.3	583	675	6 hours 12 minutes to 96 hours.	
Do	1:7,450	134	Hard	7.8	4.5	640	756	∞	
Do	[3] 1:7,500	133	do	7.8	5.0	645	673	∞	
Do	[3] 1:10,000	100	do	7.8	6.8	645	698	∞	
Do	1:17,000	59	Very soft	6.2	3.2	<50	450	1 hour to 1 hour 15 minutes.	
Do	1:60,000	17	do	6.2	4.5	<50	93	∞	
Do	[3] 1:100,000	10	Hard	7.8	7.5	645	692	∞	
Do	1:120,000	8	Very soft	6.2	5.6	<50	85	∞	
Do	[3] 1:1,000,000	1	Hard	7.8	7.5	645	695	∞	
Tannic acid	1:1,000	1,000	do	7.8	6.4	666	631	2 hours 38 minutes to 3 hours 48 minutes.	Tannery wastes.
Do	1:5,000	200	do	7.9	7.3	652	652	7 hours 26 minutes to 9 hours 26 minutes.	
Do	1:10,000	100	do	7.9	7.6	652	652	9 hours 40 minutes to 20 hours 20 minutes.	
Do	1:100,000	10	do	7.9	7.8	652	652	∞	
Tartaric acid	1:1,000	1,000	Very soft	6.2	3.2	<50	900	20 to 25 minutes	Winery wastes.
Do	1:1,000	1,000	Hard	7.8	3.9	655	727	3 hours to 3 hours 20 minutes.	
Do	1:5,000	200	do	7.8	4.6	655	700	∞	
Do	1:10,000	100	Very soft	6.2	3.6	<50	205	3 hours to 3 hours 33 minutes.	
Do	1:100,000	10	do	6.2	4.9	<50	<50	∞	

[1] Standard goldfish weight 3 to 5 grams used. Each fish in individual glass container carrying 3 liters of solution. Dissolved oxygen 6 to 7 p. p. m.; temperature maintained between 18° to 23° C. Diluent waters discussed on page —.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 4 days without any apparent injury to the fish.

[3] Initial volume of 3 liters per fish replaced by constant flow apparatus at rate of 1 liter per hour.

swelling of tissues which is very destructive to the living cells, so that although this acid does not coagulate the gill membrane and gill mucus like hydrochloric acid, it does disrupt the cells of gill membrane with disastrous results. Consequently, it is not surprising to find that acetic acid kills goldfish at pH of 5.5.

Reviewing all of the data on acid wastes, it seems that the truly acid effects must be limited largely to those acids which kill at a hydrogen-ion concentration more acid than pH 5.0; while in the cases of those acids killing at hydrogen-ion concentration less acid than pH 5.0, lethality factors other than hydrogen-ion concentration play the major part. Of course, as the hydrogen-ion concentration of the surrounding medium in which the fishes and other aquatic animals are living

Table 9.—*Survivals of 4,500 Daphnia magna in various concentrations of 5 acids found in industrial wastes* [1]

Substance	Concentration ratio by weight	Parts per million	pH		Specific conductivity $\text{mho}\times10^{-6}$ at 25° C.		Survival time	Final percent mortality
			Water	Solution	Water	Solution		
Acetic acid	1:1,333	750	7.4	4.0	470	516	5 hours	100
Do	1:2,000	500	7.4	4.6	470	491	do	100
Do	1:5,333	188	7.4	5.0	470	461	do	100
Do	1:8,000	125	7.4	5.4	470	460	24 to 72 hours	75
Citric acid	1:2,666	375	7.6	4.0	425	532	1 to 2 hours	100
Do	1:4,210	248	7.6	4.6	425	491	2 to 17 hours	100
Do	1:5,405	185	7.6	5.0	425	490	10 to 17 hours	100
Do	1:8,340	120	7.6	5.5	425	466	24 to 72 hours	40
Do	1:12,500	80	7.6	5.9	425	440	∞	0
Hydrochloric acid	1:14,545	69	7.4	4.0	452	660	1 to 4 hours	100
Do	1:15,384	65	7.4	4.5	452	650	do	100
Do	1:16,667	60	7.4	5.0	452	614	4 to 17 hours	100
Do	1:17,777	56	7.4	5.4	452	595	17 to 72 hours	80
Lactic acid	1:3,470	288	7.6	4.0	442	495	3 hours	100
Do	1:4,280	234	7.6	4.5	442	463	do	100
Do	1:5,230	191	7.6	5.0	442	443	6 to 48 hours	100
Do	1:5,880	170	7.6	5.5	442	441	26 to 72 hours	66
Sulphuric acid	1:20,000	50	7.3	3.5	324	610	1 to 3 hours	100
Do	1:26,667	38	7.3	4.0	324	484	24 hours	100
Do	1:33,333	30	7.3	4.5	324	389	do	100
Do	1:34,479	29	7.3	5.0	324	381	24 to 72 hours	100
Do	1:50,000	20	7.6	6.5	375	387	168 hours	0
Do	1:100,000	10	7.6	7.3	375	375	do	0
Do	1:1,000,000	1	7.6	7.6	375	375	do	0
Do	1:10,000,000	0.1	7.6	7.6	375	375	do	0

[1] Standard daphnia, 5 days old, from parthenogenic clones were used. For each dilution 150 animals were carried in glass jars containing 4 liters of the solution, and these jars held at 25° C. in constant temperature chamber. The diluent was soft water (see p. 406) from the same source as that in which the stock colonies of daphnia were living. A control of 150 animals in 4 liters of this water without test substance was maintained for each series.

becomes more acid than pH 7.2 to 7.4 (the normal values for most living cells), detrimental effects which may act synergistically with the specific toxic actions of the substances producing such deviations in pH must be expected.

In this connection, it must be pointed out that although the lethality limits of acid wastes as given here are applicable to immediate or intermittent pollution the cumulative effects must also be considered. When goldfish were kept in water at pH 4.5 produced by addition of small quantity of sulphuric acid, this amount of sulphuric acid, although tolerated for a few days without apparent injury to the fish, seemed definitely detrimental to goldfish in exposures longer than 2 weeks, even though some fishes can tolerate pH 4.5 in the natural habitat when this degree of acidity is produced by carbon dioxide and acids from decaying vegetation in bog and swamp lakes. It may be necessary, therefore, to add sulphuric and other acids to the lists of those producing specific toxic effects when long-time experiments in progress are completed.

Table 9 presents data concerning the effects of acids on daphnia comparable to those given in Table 8 for fishes. In the cases of daphnia which do not produce volumes of protective mucus to remove or in part buffer down the acid in the effluents to which they may be exposed, the maximal acidity of the solutions in which there was any survival by daphnia was around pH 5.4. It is significant in this connection to recall that the isoelectric point at which many proteins found in living tissue are precipitated is pH 5.5. The daphnia tests, therefore, suggest strongly that were fishes unprotected by mucus the lethal hydrogen-ion concentration for acids in general would be near pH 5.5, and explain the detrimental effects of acid wastes to the stream faunae in general even though the particular concentration may be survived by some fishes. In waters less acid than pH 5.5 the same relative lethalities of the various acids were found for daphnia as for fishes.

COMPOUNDS OF VARIOUS METALS

Compounds of many metals are rapidly lethal, because these compounds coagulate and precipitate the mucus secreted by the gills of fishes (Carpenter, 1930) and many of the proteins in the living cells. This action has been explained in the discussion of the effects of pollutants on fishes (p.154.). In addition, some metallic salts, such as ferric chloride, if present in sufficient quantities may increase the acidity of the water to a level dangerous for aquatic life and combine this hazard with that of the metallic coagulant.

Since the carbonates and phosphates of many metals are relatively insoluble in water, the hardness of waters receiving effluents containing compounds of the heavier metals is an important factor in determining the immediate lethality of such effluents to fishes, as a considerable portion of these metals may be precipitated from the effluent by the salts in the water. This factor has led to much confusion concerning the absolute limits of lethality of metallic compounds for fishes, since various observers have used different kinds of test water, ranging from distilled to very

TABLE 10.—*Influence of other salts in solution upon the toxicity of copper sulphate to gold fish* [1]

Series	Copper sulphate concentration		Sodium nitrate concentration		Calcium chloride concentration		pH		Specific conductivity mho $\times 10^{-6}$ at 25° C.		Average survival time
	Ratio by weight	Parts per million	Ratio by weight	Parts per million	Ratio by weight	Parts per million	Water	Solution	Water	Solution	
A	1:100,000	10					7.1	4.0	<50	112	2 hours 30 minutes.
B	1:100,000	10	1:2,000	500			7.1	5.7	<50	806	3 hours.
C	1:100,000	10	1:1,000	1,000			7.1	5.7	<50	1,478	3 hours 45 minutes.
D	1:100,000	10	1:500	2,000			7.1	5.8	<50	2,749	6 hours 10 minutes.
E	1:100,000	10	1:333	3,000			7.1	5.8	<50	4,020	6 hours 15 minutes.
F	1:100,000	10	1:250	4,000			7.1	6.0	<50	5,413	6 hours 30 minutes.
G	1:100,000	10	1:200	5,000			7.1	6.0	<50	6,668	6 hours 40 minutes.
H	1:100,000	10	1:167	6,000			7.1	6.4	<50	7,734	5 hours.
J	1:100,000	10	1:143	7,000			7.1	6.2	<50	9,013	5 hours 30 minutes.
K	1:100,000	10	1:125	8,000			7.1	6.3	<50	10,113	4 hours.
L	1:100,000	10	1:2,000	500	1:20,000	50	7.1	6.3	<50	1,513	5 hours 15 minutes.
M	1:100,000	10	1:1,000	1,000	1:20,000	50	7.1	6.3	<50	1,541	5 hours 30 minutes.
N	1:100,000	10	1:500	2,000	1:20,000	50	7.1	6.4	<50	2,825	6 hours 30 minutes.
O	1:100,000	10	1:333	3,000	1:20,000	50	7.1	6.5	<50	3,984	9 hours 30 minutes.
P	1:100,000	10	1:250	4,000	1:20,000	50	7.1	6.4	<50	5,350	12 hours 30 minutes.
Q	1:100,000	10	1:200	5,000	1:20,000	50	7.1	6.4	<50	6,573	12 hours 45 minutes.
R	1:100,000	10	1:167	6,000	1:20,000	50	7.1	6.5	<50	7,866	8 hours
S	1:100,000	10	1:143	7,000	1:20,000	50	7.1	6.5	<50	8,763	7 hours.
T	1:100,000	10	1:125	8,000	1:20,000	50	7.1	6.5	<50	10,338	Do.
U			1:333	3,000			7.1	7.3	<50	4,221	(2).
V			1:250	4,000			7.1	6.7	<50	5,350	80 hours.
W			1:200	5,000			7.1	6.8	<50	6,620	37 hours.
X			1:167	6,000			7.1	6.9	<50	7,798	6 hours 30 minutes.

[1] Glass distilled water was used in these tests. Other conditions as described in table 8.
[2] Survival greater than 4 days, fish apparently unaffected.

hard. Unless the hardness of the water in question and particularly the amounts of carbon dioxide, both fixed and free, be known the limits of lethality of any particular metallic compound in any given water are difficult to estimate even though there may be ample data on the specific toxicity of that compound. Besides, the factors of synergy and antagonism between the metallic compound itself and other compounds in the water or effluent which do not precipitate this metallic compound must be considered. Examples of this action are presented in Table 10 for copper sulphate.

It may be noted in this table that the addition of progressively larger quantities of sodium nitrate to the copper sulphate solution increased the survival time of fishes from 2 hours 30 minutes to 6 hours 40 minutes, up to a concentration of 1 : 200 for sodium nitrate. Beyond that concentration the osmotic action of the sodium nitrate became unfavorable (see osmotic pressure, p.154.) and the survival time decreased with a further increase of sodium nitrate, i.e., sodium nitrate, which does not precipitate copper, both increased and decreased the survival time of fishes exposed to the lethal action of same amounts of copper sulphate according to the concentration of sodium nitrate present. The second half of this table shows the marked increase in survival time from 2 hours and 30 minutes in the copper sulphate alone to 12 hours 45 minutes in the same strength of copper sulphate solution to which both sodium nitrate and calcium chloride were added. The amount of calcium chloride used was very small, namely, 50 p.p.m., and in this concentration no precipitate formed, yet this quantity of calcium chloride greatly enhanced the protective action of the sodium nitrate against the lethal action of copper sulphate.

These discussions of the precipitation of metallic compounds out of effluents and of the synergistic and antagonistic actions of compounds which do not precipitate the particular metal in question show, both specifically in the case of effluents carrying compounds of metals and in general in the case of all stream pollutants carrying chemical wastes, that field examinations of local conditions, analyses both of the effluents and the water receiving these effluents, and bioassays of the mixtures of the two must be made before the lethal limits of such wastes can be determined.

In Table 11 summarized data from a large series of tests covering the lethality limits of certain compounds of eight metals found in commercial effluents are presented. From these data the expected lethal ranges and the relative lethality of these eight compounds in various types of water, together with the changes in salt balance and relative acidity produced by these compounds, may be estimated if uncomplicated by substances other than those normally found in stream water, or by excessive amounts of those substances.

Another hazard from wastes containing compounds of various metals is the cumulative effect. Even though precipitated out, compounds carrying these metals, as long as they remain in the stream, on the bank, or

TABLE 11.—*Survivals of 850 goldfish in various concentrations of salts of 8 metals found in industrial wastes* [1]

Substance	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho $\times 10^{-6}$ at 25° C.		Survival time [2]	Constituent of—
				Water	Solution	Water	Solution		
Aluminum potassium sulphate	[3] 1:1,000	1,000	Hard	7.8	5.5	678	1,247	1 to 10 hours	Tannery wastes.
Do	[3] 1:10,000	100	do	7.8	6.8	678	736	12 hours to ∞	
Do	[3] 1:100,000	10	do	7.8	7.6	678	584	∞	
Do	1:1,000,000	1	do	7.8	7.7	678	677	∞	
Cobaltous chloride	1:1,000	1,000	Very soft	6.2	6.6	<50	1,906	28 to 29 hours	Electroplating wastes.
Do	1:1,000	1,000	Hard	7.8	7.2	647	2,265	30 to 31 hours	
Do	1:10,000	100	Very soft	6.2	6.5	<50	308	168 hours to ∞	
Cupric sulphate	[3] 1:1,000	1,000	Glass distilled	7.0	5.6	<50	1,123	1 to 2 hours	Copper and brass industries wastes.
Do	[3] 1:1,000	1,000	Hard	7.8	6.8	690	1,348	1 hour to 2 hours 20 minutes.	
Do	[3] 1:10,000	100	do	7.8	7.0	671	637	3 hours to 11 hours	
Do	1:10,000	100	do	7.9	7.0	635	637	3 to 41 hours	
Do	[3] 1:100,000	10	do	7.9	7.6	635	671	11 to 72 hours	
Do	1:100,000	10	do	7.9	7.7	635	700	48 hours to ∞	
Do	[3] 1:1,000,000	1	do	7.9	7.5	635	640	72 hours to ∞	
Ferrous sulphate	[3] 1:1,000	1,000	do	7.7	6.4	594	1,307	2 to 10 hours	Wire and tin-plate mills wastes.
Do	[3] 1:10,000	100	do	7.7	6.7	594	649	∞	
Do	[3] 1:100,000	10	do	7.7	7.4	594	598	∞	
Do	[3] 1:1,000,000	1	do	7.7	7.6	594	600	∞	
Ferric chloride	1:1,000	1,000	Very soft	6.2	2.4	<50	2,156	1 hour to 1 hour 10 minutes.	
Do	1:10,000	100	do	6.2	3.4	<50	334	1 hour to 1 hour 20 minutes.	
Do	1:10,000	100	Hard	7.8	5.5	647	700	∞	
Do	1:100,000	10	Very soft	6.2	5.0	<50	<50	∞	
Lead nitrate	[3] 1:1,000	1,000	Hard	7.8	6.4	678	2,500	2 to 3 hours	Mining and smelting wastes.
Do	[3] 1:10,000	100	do	7.8	6.8	678	775	80 hours to ∞	
Do	[3] 1:100,000	10	do	7.8	7.4	678	678	∞	
Do	[3] 1:1,000,000	1	do	7.8	7.5	678	673	∞	
Nickelous chloride	1:1,000	1,000	Very soft	6.3	6.3	<50	1,876	6 hours to 18 hours 30 minutes.	Electroplating wastes.
Do	1:1,000	1,000	Hard	7.8	7.4	647	2,218	12 to 18 hours	
Do	1:10,000	100	Very soft	6.3	6.3	<50	253	19 hours 25 minutes to 50 hours 25 minutes.	
Do	1:100,000	10	do	6.5	6.5	<50	95	200 to 210 hours	
Stannous chloride	1:1,000	1,000	Very soft	6.4	3.5	<50	2,380	1 hour to 1 hour 30 minutes.	Brass industries wastes.
Do	1:1,000	1,000	Hard	7.8	3.8	647	1,609	4 to 5 hours	
Zinc sulphate	[3] 1:1,000	1,000	do	7.8	7.2	678	1,538	1 to 4 hours	Mining and smelting wastes.
Do	[3] 1:10,000	100	do	7.8	7.6	678	780	∞	
Do	[3] 1:100,000	10	do	7.8	7.6	678	678	∞	
Do	[3] 1:1,000,000	1	do	7.8	7.6	678	678	∞	

---

[1] Experimental conditions as in table 8.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 4 days without any apparent injury to the fish.

[3] Initial volume of 3 liters per fish replaced by constant flow apparatus at rate of 1 liter per hour.

on the floor of the stream, are subject to re-solution if conditions in the waters change (in many cases only slightly), and to ingestion by fish and other aquatic animals during feeding.

The case of lead salts will suffice for example. Carpenter in a series of papers (1924, 1925, 1926, 1927, and 1930) has shown that the lethal action of lead compounds on fishes in cases where the more soluble lead salts are present is due to the precipitation of mucus and proteins in and on the gills by the lead ion, and that the absorption and, therefore, the internal poisoning of fishes by lead compounds under these conditions is negligible during moderately short exposures. This conclusion has been confirmed by Behrens (1925) using a very delicate test (the radio-activity of an isotope of lead) which demonstrated the distribution of lead throughout the body of the fish.

However, the writer in long-time experiments in which goldfish were kept in glass containers, the bottoms of which were covered with finely powdered lead ore (lead sulphide, which is very insoluble in water), found cumulative effects which resulted in the death of fish after 61 days of such exposure, although there were no casualties in the controls carried under identical conditions except for the presence of lead ore. Although these experiments which are being followed further do not in themselves prove that the cumulative action was that of lead alone or of lead in conjunction with some other substance (i.e., traces of other substances in the ore may have been responsible for these cumulative effects), these experiments do show that precipitated and insoluble substances on the bottom of the stream can be a definite pollution hazard over a long period of time due to cumulative action. Again, the writer has found that insoluble zinc ore (zinc sulphide) carried by a stream and deposited on flats adjacent to the main stream bed during high water when exposed to the actions of sunlight, air, and moisture produced quantities of zinc sulphate, a freely soluble and definitely toxic compound which was leached back through into the stream subsequently by rains and high waters (Ellis, 1932).

The synergistic physiological action of many compounds is well known, and the work of Macht and Leach (1930) on goldfish and other types of animals may be cited in this connection, as these writers found that two or more octylic alcohols were synergistic in their action on the respiratory and neuromuscular mechanisms of animals.

MISCELLANEOUS COMPOUNDS

In Tables 12 and 13 the relative toxicities of 13 other substances found in industrial wastes or otherwise constituting stream pollution hazards are presented. The changes in hydrogen-ion concentration and salt balance are shown, as in the tables giving the data on acids and metallic compounds. It may be seen, however, that the substances listed in Tables 12 and 13 do not kill because of changes either in pH or salt balance, i.e., in these two tables are included representatives of those substances which, if detrimental, are so because of specific toxic action after entering the body of the fish. Phenol produces paralysis of the neuromuscular mechanisms and hemolyzes the blood; potassium xanthate in high dilution acts after several days on the gastrointestinal tract; amyl alcohol quickly induces a semiparalyzed condition with marked incoordination, yet the fish may live for days in this state before dying; and sodium selenite causes a slow decline in the general activities of the fish.

TABLE 12.—*Survival of goldfish in solutions of sulphur and selenium compounds*[1]

Substance	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho×10⁻⁶ at 25° C.		Survival time[2]	Constituent of—
				Water	Solution	Water	Solution		
Ammonium sulphide	[3] 1:1,000	1,000	Hard	7.7	7.9	681	938	15 minutes to 1 hour 30 minutes.	Sewage and organic wastes.
Do	[3] 1:10,000	100	do	7.7	7.8	681	735	72 hours to ∞	
Do	[3] 1:100,000	10	do	7.7	7.7	681	681	∞	
Do	[3] 1:1,000,000	1	do	7.7	7.7	681	681	∞	
Hydrogen sulphide	[3] 1:1,000	1,000	do	7.8	6.5	670	734	45 minutes to 1 hour	Do.
Do	[3] 1:10,000	100	do	7.8	7.3	670	676	3 to 4 hours	
Do	[3] 1:100,000	10	do	7.8	7.6	670	661	96 hours to ∞	
Do	[3] 1:1,000,000	1	do	7.8	7.7	670	670	∞	
Sodium sulphite	[3] 1:1,000	1,000	do	7.8	7.6	676	2,017	3 to 72 hours	Paper-pulp-mill wastes.
Do	[3] 1:10,000	100	do	7.8	7.6	676	864	96 hours to ∞	
Do	[3] 1:100,000	10	do	7.8	7.6	676	678	∞	
Do	[3] 1:1,000,000	1	do	7.8	7.8	676	677	∞	
Sodium selenite	1:1,000	1,000	Very soft	6.4	7.5	<50	753	1 hour to 1 hour 30 minutes.	Certain soils.
Do	1:1,000	1,000	Hard	7.8	7.4	647	1,141	1 hour to 2 hours 10 minutes.	
Do	1:10,000	100	Very soft	6.4	7.5	<50	107	20 hours 56 minutes to ∞.	
Do	1:10,000	100	Hard	7.8	7.7	647	723	8 hours to 19 hours 30 minutes.	
Do	1:100,000	10	Very soft	6.4	7.3	<50	<50	98 to 144 hours	

[1] Conditions of experiments described in table 8.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 4 days without any apparent injury to the fish.

[3] Constant flow as described in table 8.

TABLE 13.—*Survivals of goldfish in solutions of various chemicals found in industrial wastes* [1]

Substance	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho×10^{-6} at 25° C.		Survival time [2]	Constituent of
				Water	Solution	Water	Solution		
Ammonium carbonate	[3] 1:1,000	1,000	Hard	7.7	8.1	718	2,644	1 to 2 hours	Organic wastes, gashouse wastes.
Do.	[3] 1:10,000	100	do.	7.7	7.8	718	956	4 to 10 hours	
Do.	[3] 1:100,000	10	do.	7.7	7.7	718	727	∞	
Do.	[3] 1:1,000,000	1	do.	7.7	7.7	718	718	∞	
Amyl alcohol (iso)	1:1,000	1,000	do.	7.8	7.8	647	666	17 to 71 hours	Distillery wastes.
Do.	1:10,000	100	do.	7.8	7.8	647	647	82 hours-∞	
Do.	1:100,000	10	do.	7.8	7.8	647	647	94 hours-∞	
Do.	1:1,000,000	1	do.	7.8	7.8	647	647	161 hours-∞	
Bromine	1:1,000	1,000	do.	8.0	6.7	646	673	20 to 40 minutes	Chemical industries wastes.
Do.	1:2,500	400	do.	8.0	7.4	646	659	30 minutes to 1 hour 10 minutes.	
Do.	1:10,000	100	do.	8.0	7.6	646	649	1 hour to 3 hours 25 minutes.	
Do.	1:50,000	20	do.	8.0	8.0	646	646	15 to 96 hours	
Do.	1:100,000	10	do.	8.0	8.0	646	646	∞	
Chlorine	1:10,000	100	do.	7.9	7.4	653	640	54 minutes to 1 hour 30 minutes.	Do.
Do.	1:100,000	10	do.	7.9	7.9	653	653	5 hours 15 minutes to 48 hours.	
Do.	1:500,000	2	do.	7.9	7.9	653	653	17 to 48 hours	
Do.	1:1,000,000	1	do.	7.9	7.9	653	653	96 hours to ∞	
Cresylic acid	1:1,000	1,000	do.	7.8	7.8	639	654	14 to 30 minutes	Livestock disinfectants and flotation wastes.
Do.	1:10,000	100	do.	7.8	7.8	639	641	5 to 31 hours	
Do.	1:100,000	10	do.	7.8	7.8	639	640	46 to 96 hours	
Do.	1:1,000,000	1	do.	7.8	7.8	645	645	93 to 120 hours	
Do.	1:10,000,000	0.1	do.	7.8	7.8	645	645	120 hours to ∞	
Phenol	[3] 1:1,000	1,000	do.	7.8	7.6	690	587	15 to 30 minutes	Livestock disinfectants and gashouse wastes.
Do.	[3] 1:10,000	100	do.	7.8	7.6	690	593	60 to 72 hours	
Do.	[3] 1:100,000	10	do.	7.8	7.8	690	690	72 hours to ∞	
Do.	[3] 1:1,000,000	1	do.	7.8	7.8	690	690	∞	
Potassium xanthate	[3] 1:3,125	320	do.	8.1	8.8	669	900	5 to 24 hours	Mine flotation wastes.
Do.	[3] 1:6,250	160	do.	8.1	8.6	669	776	12 to 24 hours	
Do.	[3] 1:12,500	80	do.	8.1	8.4	669	731	12 to 36 hours	
Do.	[3] 1:100,000	10	do.	8.1	8.1	669	669	48 to 96 hours	
Do.	[3] 1:10,000,000	0.1	do.	8.1	8.1	669	669	4 to 5 days	
Sodium chlorate	1:1,000	1,000	do.	8.0	7.7	645	753	∞	
Sodium fluoride	1:1,000	1,000	Very soft	6.4	6.4	<50	2,162	12 to 29 hours	Distillery wastes and certain soils.
Do.	1:1,000	1,000	Hard	7.8	7.0	647	2,346	60 to 102 hours	
Do.	1:10,000	100	do.	7.8	7.3	647	943	∞	

[1] Condition of experiment described in table 8.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 4 days without apparent injury to the fish.

[3] Constant flow as described in table 8.

The specifically toxic substances which do enter the body of the fish can be grouped therefore according to their specific pathological and pharmacological effects much as in the case of man; and these poisons which do enter the body of the fish constitute important pollution hazards in high dilution, since the high dilutions may be tolerated by the fish for a short time without apparent harmful results. Consequently, the compounds in this miscellaneous group must be considered individually in evaluating pollution hazards.

LETHAL LIMITS OF 114 SUBSTANCES WHICH MAY BE FOUND IN STREAM POLLUTANTS

In this section summaries of the lethal limits of individual substances, together with bibliographic references and statements concerning the test animals used and conditions of the experiments, are presented. These data have been massed from the literature and from the writer's own experiments (M.M.E.). In these summaries no attempt has been made to include all existing references, but instead sufficient data to give a definite idea of the relative lethality of the substance in question under conditions which may be encountered in the streams of the United States. Only fresh-water fishes and fresh-water organisms have been considered. It is hoped that these summaries will prove helpful in evaluating specific pollution conditions when used in conjunction with the data presented in preceding sections. However, as has been pointed out repeatedly in this paper, arbitrary application of lethality data to specific pollution problems is absolutely impossible owing to the many limiting factors, various of which have been discussed in previous sections.

Any student of the toxicity of the components of industrial and municipal effluents to fishes and other aquatic life will find the pioneer work of Penny and Adams (1863) and of Weigelt, Saare, and Schwab (1885) starting points for the investigations of the commoner substances. However, much progress has been made in the study of the factors limiting the action of many

TABLE 13.—*Survivals of goldfish in solutions of various chemicals found in industrial wastes* [1]

Substance	Concentration ratio by weight	Parts per million	Diluent water	pH		Specific conductivity mho$\times 10^{-6}$ at 25° C.		Survival time [2]	Constituent of
				Water	Solution	Water	Solution		
Ammonium carbonate	[3] 1:1,000	1,000	Hard	7.7	8.1	712	2,944	1 to 2 hours	Organic wastes, gashouse wastes.
Do	[3] 1:10,000	100	do	7.7	7.8	713	956	4 to 10 hours	
Do	[3] 1:100,000	10	do	7.7	7.7	718	727	∞	
Do	[3] 1:1,000,000	1	do	7.7	7.7	718	718	∞	
Amyl alcohol (iso)	1:1,000	1,000	do	7.8	7.8	647	666	17 to 71 hours	Distillery wastes.
Do	1:10,000	100	do	7.8	7.8	647	647	82 hours-∞	
Do	1:100,000	10	do	7.8	7.8	647	647	94 hours-∞	
Do	1:1,000,000	1	do	7.8	7.8	647	647	161 hours-∞	
Bromine	1:1,000	1,000	do	8.0	6.7	646	673	20 to 40 minutes	Chemical industries wastes.
Do	1:2,500	400	do	8.0	7.4	646	650	30 minutes to 1 hour 10 minutes.	
Do	1:10,000	100	do	8.0	7.6	646	649	1 hour to 3 hours 25 minutes.	
Do	1:50,000	20	do	8.0	8.0	646	646	15 to 96 hours	
Do	1:100,000	10	do	8.0	8.0	646	646	∞	
Chlorine	1:10,000	100	do	7.9	7.4	653	640	54 minutes to 1 hour 30 minutes.	Do.
Do	1:100,000	10	do	7.9	7.9	653	653	5 hours 15 minutes to 48 hours.	
Do	1:500,000	2	do	7.9	7.9	653	653	17 to 48 hours	
Do	1:1,000,000	1	do	7.9	7.9	653	653	96 hours to ∞	
Cresylic acid	1:1,000	1,000	do	7.8	7.8	639	654	14 to 30 minutes	Livestock disinfectants and flotation wastes.
Do	1:10,000	100	do	7.8	7.8	639	641	5 to 31 hours	
Do	1:100,000	10	do	7.8	7.8	639	640	46 to 96 hours	
Do	1:1,000,000	1	do	7.8	7.8	645	645	93 to 120 hours	
Do	1:10,000,000	0.1	do	7.8	7.8	645	645	120 hours to ∞	
Phenol	[3] 1:1,000	1,000	do	7.8	7.6	690	587	15 to 30 minutes	Livestock disinfectants and gashouse wastes.
Do	[3] 1:10,000	100	do	7.8	7.6	690	593	60 to 72 hours	
Do	[3] 1:100,000	10	do	7.8	7.8	690	690	72 hours to ∞	
Do	[3] 1:1,000,000	1	do	7.8	7.8	690	690	∞	
Potassium xanthate	[3] 1:3,125	320	do	8.1	8.8	669	900	5 to 24 hours	Mine flotation wastes.
Do	[3] 1:6,250	160	do	8.1	8.6	669	776	12 to 24 hours	
Do	[3] 1:12,500	80	do	8.1	8.4	669	731	12 to 36 hours	
Do	[3] 1:100,000	10	do	8.1	8.1	669	669	48 to 96 hours	
Do	[3] 1:10,000,000	0.1	do	8.1	8.1	669	669	4 to 5 days	
Sodium chlorate	1:1,000	1,000	do	8.0	7.7	645	753	∞	
Sodium fluoride	1:1,000	1,000	Very soft	6.4	6.4	<50	2,162	12 to 29 hours	Distillery wastes and certain soils.
Do	1:1,000	1,000	Hard	7.8	7.0	647	2,346	60 to 102 hours	
Do	1:10,000	100	do	7.8	7.3	647	943	∞	

[1] Condition of experiment described in table 8.

[2] Minimal and maximal survival times as found in these experiments. Infinity sign indicates survival greater than 4 days without apparent injury to the fish.

[3] Constant flow as described in table 8.

The specifically toxic substances which do enter the body of the fish can be grouped therefore according to their specific pathological and pharmacological effects much as in the case of man; and these poisons which do enter the body of the fish constitute important pollution hazards in high dilution, since the high dilutions may be tolerated by the fish for a short time without apparent harmful results. Consequently, the compounds in this miscellaneous group must be considered individually in evaluating pollution hazards.

LETHAL LIMITS OF 114 SUBSTANCES WHICH MAY BE FOUND IN STREAM POLLUTANTS

In this section summaries of the lethal limits of individual substances, together with bibliographic references and statements concerning the test animals used and conditions of the experiments, are presented. These data have been massed from the literature and from the writer's own experiments (M.M.E.). In these summaries no attempt has been made to include all existing references, but instead sufficient data to give a definite idea of the relative lethality of the substance in question under conditions which may be encountered in the streams of the United States. Only fresh-water fishes and fresh-water organisms have been considered. It is hoped that these summaries will prove helpful in evaluating specific pollution conditions when used in conjunction with the data presented in preceding sections. However, as has been pointed out repeatedly in this paper, arbitrary application of lethality data to specific pollution problems is absolutely impossible owing to the many limiting factors, various of which have been discussed in previous sections.

Any student of the toxicity of the components of industrial and municipal effluents to fishes and other aquatic life will find the pioneer work of Penny and Adams (1863) and of Weigelt, Saare, and Schwab (1885) starting points for the investigations of the commoner substances. However, much progress has been made in the study of the factors limiting the action of many pollutants, since these experiments and the lethality values given by these earlier observers have been quoted too often without regard to more recent findings.

Important advances have been made in the measurement of stream-pollution hazards of particular effluents through the isolation from these effluents of various compounds of highly toxic nature, which compounds were formerly overlooked or disregarded because the actual amounts of these substances present in the effluent were quite small. These highly toxic compounds, some of which are volatile or may otherwise disappear from the effluent after a time, have been responsible for the wide discrepancies in lethality of the waste as a whole as reported by different observers working on the same type of effluent.

In the present list of substances which may be participants in stream-pollution problems, several compounds have been included which are little known except to the professional chemist, although the harmful properties of these substances have been demonstrated in connection with certain industrial effluents. The list of these less familiar compounds will undoubtedly grow, both as investigations of particular effluents are completed and as chemical engineering produces new commercial processes. The long series of dyes, many of which are highly toxic to aquatic life, used in industries and arts have not been included in this list of possible stream pollutants because the dye wastes are largely mordant, bleach and processing liquors containing only traces of the dyestuffs themselves which industry has found too costly to waste. The conservation of these expensive dye compounds by the manufacturing concerns has been very apparent to the writer in several recent investigations of plants dyeing fabric, paper, and leather.

ACETIC ACID, $CH_3 \cdot COOH$. Vinegar acid.

Beet-sugar pulp waste; some winery wastes; soured fruit wastes; and vinegar works. Penny and Adams (1863), 114 p.p.m. killed minnows in 20 hours, but 286 p.p.m. not fatal to goldfish; M.M.E., 100 p.p.m. in hard water killed some goldfish, Carassius auratus, and 125 p.p.m. killed cladocerans, Daphnia magna, in 24-72 hours.

ACETONE, $(CH_3)_2CO$

Gas and coal-tar wastes; paint and chemical industries. Shelford (1917), 14,250-15,050 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis.

ACRIDINE, $C_6H_4(N \cdot CH)C_6H_4$

Gas tar oils; dye industries; water sterilization. Adams (1927), 5 p.p.m. in Nile River water killed cladocerans, Daphnia sp., and copepods, Cyclops sp.

ALCOHOLS

See amyl alcohol, butyl alcohol, ethyl alcohol, methyl alcohol, and octyl alcohol.

ALUMINIUM AMMONIUM SULPHATE, $Al(NH_4)(SO_4)_2$. Ammonium alum.

Dye works and cloth printing industries. Weigelt, Saare, and Schwab (1885), 523 p.p.m. in tap water caused large trout to float on side after 10 hours' exposure.

ALUMINIUM POTASSIUM SULPHATE, $AlK(SO_4)_2$. Alum or potassium alum.

Dye works; tanneries; leather works. Penny and Adams (1863), 250 p.p.m. killed goldfish and minnows; Weigelt, Saare, and Schwab (1885), 544 p.p.m. in tap water killed California salmon in 6 hours, and medium trout in 15 hours; M.M.E., 1,000 p.p.m. in hard water killed goldfish, Carassius auratus in 1-10 hours, 100 p.p.m. killed goldfish in 12-96 hours, although some survived this strength over 100 hours.

ALUMS

See aluminium ammonium sulphate, aluminium potassium sulphate, and ferric potassium sulphate.

AMMONIA

See ammonium hydroxide.

AMMONIUM CARBONATE, $(NH_4)_2CO_3$

Gas wastes; wool-washings; tannery effluents. Clark and Adams (1913), 155-197 p.p.m. fatal to shiners and carp in a few minutes to a few hours; Shelford (1917), 600-800 p.p.m. killed orange-spotted sunfish, Lepomis humilis in 1 hour; M.M.E., 100 p.p.m. in hard water killed goldfish in 4-10 hours, 48 p.p.m. in 6 days, 10 p.p.m. tolerated for more than 100 hours without apparent effect.

AMMONIUM CHLORIDE, NH_4Cl. Sal ammoniac.

Gas wastes; various chemical wastes. Clark and Adams (1913), 180 p.p.m. in tap water, no effect on shiners or carp; Wells (1915b), 535 p.p.m. in tap water killed bluegills, Lepomis pallidus in 4 hours 45 minutes, and same amount in distilled water killed bluegills in 18 days; Shelford (1917), 700-800 p.p.m. killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Powers (1917), 1,712 p.p.m. in distilled water killed goldfish, Carassius auratus, in 6-18 hours; M.M.E. 268 p.p.m. in hard water, killed goldfish in 6 days; 535 p.p.m. killed cladocerans, Daphnia magna, in 6 hours.

AMMONIUM FERROCYANIDE $(NH_4)_4Fe(CN)_6$

Gas wastes. Shelford (1917), 150-200 p.p.m. killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

AMMONIUM HYDROXIDE, NH_4OH

Wool washings; chemical wastes; gas wastes. Since ammonia gas NH_3 unites readily on mixing with water forming the hydroxide, these two compounds are considered together here. Clark and Adams (1913), 9.4 p.p.m. (ammonium hydroxide) did not kill shiners, carp, and large suckers but 13 p.p.m. killed all these fishes, 20 p.p.m. being fatal in 15 minutes; Belding (1928), 6.25 p.p.m. killed brook trout in 24 hours; Weigelt, Saare, and Schwab (1885), 250 p.p.m. (ammonia) killed trout in 2 hours; Shelford (1917), 7-8 p.p.m. (ammonia) in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; M.M.E., 2-2.5 p.p.m. (ammonia) in hard water fatal to goldfish, Carassius auratus, and yellow-perch, Perca flavescens, in 24 hours to 4 days.

AMMONIUM NITRATE, NH_4NO_3

Chemical industries; manufacture of explosives; in fertilizers. Wells (1915b), 800 p.p.m. in tap water killed bluegills, Lepomis pallidus, in 3.9 hours; in distilled water lethal to bluegills in 16 days; Powers (1917), 4,545 p.p.m. in distilled water killed goldfish, Carassius auratus, in 90 hours.

AMMONIUM SULPHATE, $(NH_4)_2SO_4$

Chemical wastes; gas wastes; fertilizers. Wells (1915b), 66 p.p.m. in tap water killed bluegills, Lepomis pallidus, in 3 hours, 30 minutes, in distilled water lethal to bluegills in 17 days; Shelford (1917), 420-500 p.p.m. in tap water killed Lepomis humilis, in 1 hour; M.M.E., 264 p.p.m. in hard water killed goldfish, Carassius auratus, in 6 days or less.

AMMONIUM SULPHIDE, $(NH_4)_2S_x$

Chemical wastes; in wastes from some beet-sugar processes; in some gas wastes. M.M.E., 100 p.p.m. in hard water killed goldfish, Carassius auratus, in 72 hours, 10 p.p.m. in hard water, goldfish survived more than 100 hours of exposure.

AMMONIUM THIOCYANATE, NH_4CNS.
Rhodanammonium

Gas wastes; chemical industries. Shelford (1917), 280-300 p.p.m. in tap water killed orange-spotted sunfish in 1 hour; Demyanenko (1931), 200 p.p.m. lethal for fish.

AMYL ALCOHOL (iso), $C_5H_{11}OH$

Chemical wastes; in some distillery wastes. M.M.E., 100 p.p.m. in hard water killed goldfish, Carassius auratus, in 82 hours, 1 p.p.m. killed goldfish in 161 hours.

ANILINE, $C_6H_5NH_2$

Dye wastes; chemical industries; gas wastes. Shelford (1917), 1,020-1,122 p.p.m. killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Demyanenko (1931), 250 p.p.m. lethal for fishes.

ARSENIC TRIOXIDE, white arsenic

See sodium arsenate.

BARIUM CHLORIDE, $BaCl_2$

Some chemical wastes; some alkaline pools. Powers (1917), 5,000 p.p.m. in distilled water killed goldfish, Carassius auratus, in 12-17 hours.

BENZOIC ACID, $C_6H_5\cdot COOH$

Gas wastes. Shelford (1917), 550-570 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; M.M.E., 200 p.p.m. in hard water killed goldfish in 7-96 hours.

BLUE VITRIOL

See cupric sulphate.

BROMINE, Br_2

Chemical industries; bromine plant wastes. Penny and Adams (1863), 29 p.p.m. killed minnows and goldfish; M.M.E., 20 p.p.m. in hard water killed goldfish in 15-96 hours, 10 p.p.m. in soft water lethal for cladocerans, Daphnia magna.

BUTYL ALCOHOL (iso), C_4H_9OH

Paint and varnish solvents; some chemical industries. Powers (1917), 250 p.p.m. in distilled water killed goldfish, Carassius auratus, in 7-20 hours.

CADMIUM CHLORIDE, $CdCl_2$

Pigment works; calico printing; chemical wastes. Powers (1917), 0.0165 p.p.m. in distilled water killed goldfish in 8 hours 40 minutes to 18 hours.

CADMIUM SULPHATE, $CdSO_4$

Same sources as cadmium chloride. Carpenter (1927), 1,042 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in 3 hours.

CALCIUM CHLORIDE, $CaCl_2$

Wastes from bromine and salt works; in waters from oil wells; antidust road surfacing. Wells (1915b) 555 p.p.m. in tap water caused pathological degeneration of tail fin of rock-bass, Ambloplites rupestris, in an exposure of 1 week; Garrey (1916), 2,775 p.p.m. in distilled water killed straw-colored minnows, Notropis blennius, in 2-4 days, but this species did not succumb to 277 p.p.m. in distilled water in 5-7 weeks; Powers (1917), 7,752 p.p.m. in distilled water killed goldfish, Carassius auratus, in 22-27 hours; Wiebe, Burr, and Faubion (1934), 5,000 p.p.m. killed golden shiners, Notemigonus crysoleucas, in 143 hours.

CALCIUM HYDROXIDE, $Ca(OH)_2$. Lime.

Tannery-wastes; leather works. Weigelt, Saare, and Schwab (1885), 700 p.p.m. in tap water killed trout in 26 minutes; Marsh (1907), 18 p.p.m. (as calcium oxide) fatal to trout fry.

CALCIUM NITRATE, $Ca(NO_3)_2$

Some chemical wastes. Powers (1917), 6,061 p.p.m. in distilled water killed goldfish, in 43-48 hours.

CALCIUM OXIDE, CaO. Unslaked lime.

Forms calcium hydroxide, q.v., immediately on addition to water.

CARBON BISULPHIDE, CS_2

Gas wastes; as a solvent in various chemical industries. Weigelt, Saare and Schwab (1885), a 7 minute exposure to 5,000 p.p.m. killed trout 2 days later; Shelford (1917), 100-127 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

CARBON DIOXIDE, CO_2

From sewage and any decomposing organic wastes (see section on carbon dioxide in natural waters). Wells (1918), 113,925-115,900 p.p.m. in tap water killed fresh-water fishes.

CARBON MONOXIDE, CO

Motorboat exhausts; in water returned to streams from cooling systems of engines using fuel oil if mixed with exhaust gases. Wells (1918), 1,160 p.p.m. in tap water killed straw-colored minnow, Notropis blennius, in 1 hour; the blunt-nosed minnow, Pimephales notatus, in 1 hour 55 minutes; the orange-spotted sunfish, Lepomis humilis, in 5 hours 40 minutes; the green sunfish, Lepomis cyanellus, in 6 hours; 11,314 p.p.m. under same conditions killed the black bullhead, Ameiurus melas, in 9 hours 55 minutes.

CHLORAMINE, NH_2Cl

Water purification systems either as chloramine or formed from small quantities of ammonia and chlorine in the water. Coventry, Shelford, and Miller (1935), 0.3-0.4 p.p.m. in tap water killed trout fry at once, and 0.06 p.p.m., trout fry in 48 hours; 0.4 p.p.m. killed sunfish and bullheads; 0.76 p.p.m. hardy minnows; and 1.2 p.p.m., large carp and bullheads.

CHLORAMINE-T, $CH_3{\cdot}C_6H_4SO_2NNaCl$

Water purification systems. Adams (1927), 5 p.p.m. in Nile River water killed cladocerans, Daphnia sp., and copepods, Cyclops sp.

CHLORINE, Cl_2

Water purification systems; various chemical wastes. Adams (1927), 2 p.p.m. in Nile River water killed cladocerans, Daphnia sp., and copepods, Cyclops sp.; Davis (1934), 1 p.p.m. killed coarse fish; M.M.E., 1 p.p.m. in hard water killed goldfish, Carassius auratus, in 96 hours; and 0.5 p.p.m. in soft water killed cladocerans, Daphnia magna, in 72 hours or more.

CHLORIDE OF LIME, or bleaching powder.

This substance is of uncertain composition as usually found. Its lethal properties as regards aquatic life are largely dependent upon the amount of chlorine which is liberated when the bleaching powder is added to the water. The potential amount of chlorine available in the sample under question must be known before its toxicity can be estimated. Much confusion ists in the previous reports because of that fact and consequently no limits are given here.

CHROMIC ACID, H_2CrO_4

Chrome tannery wastes. M.M.E., 100 p.p.m. in hard water did not kill goldfish, Carassius auratus, in 100 hours' exposure; the same amount in very soft water killed goldfish in 30-35 minutes.

CITRIC ACID, $(COOH)CH_2C(OH)(COOH)CH_2COOH$

Wastes from industries using citrus fruits. M.M.E., 894 p.p.m. in hard water killed goldfish, Carassius auratus, in 4-28 hours; 625 p.p.m. in hard water was not lethal to goldfish in 100 hours exposure; 120 p.p.m. in soft water killed cladocerans, Daphnia magna, in 24-72 hours.

COBALTOUS CHLORIDE, $CoCl_2$

Pigment works; chemical industries. M.M.E., 1,000 p.p.m. in hard water killed goldfish, Carassius auratus, in 30-32 hours; 10 p.p.m. in soft water fatal to some goldfish in 168 hours; others survived exposure to this amount for longer periods.

COPPER, Cu_2

See various cupric compounds. As Moore and Kellerman (1905) have pointed out and as is discussed here under the action of heavy metals, the amount of copper required to produce lethal results varies greatly with the water, and particularly the carbonates. These writers state that "in water containing carbonates, if the amount of dissolved CO_2 is very low the basic carbonate of copper formed may be considered insoluble; if, however, the water should contain a fair amount of CO_2 it would bring the copper carbonate at least partially into solution." Copper compounds are used as algicides and occur in many industrial wastes.

CRESOL, general formula $C_6H_4(OH)(CH_3)$

A mixture of the various isomeric compounds of this group. The mixture and the individual compounds occur in gas wastes in varying proportions and the mixture is used in various sheep dips and other preparations for the disinfecting of livestock. Both gas wastes and dipping vats have on occasion been the sources of stream pollution by this substance. This mixture and two of the component isomers are discussed below.

CRESOL

Adams (1927), 10 p.p.m. in Nile River water killed cladocerans, Daphnia sp., and copepods, Cyclops sp.; Demyanenko (1931), 17-20 p.p.m. lethal for fishes.

ORTHOCRESOL

Shelford (1917), 55-65 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; M.M.E., 10-20 p.p.m. in hard water killed goldfish, Carassius auratus, in 3-5 days.

PARACRESOL

Shelford (1917), 80-90 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Southgate, Pentelow, and Bassindale (1933), 6.2 p.p.m. in tap water caused trout, Salmo irideus, to float helpless on their backs in 1 hour 40 minutes, if the water carried circa 10 p.p.m. of dissolved oxygen and in 13 minutes if the water carried only 3 p.p.m. dissolved oxygen.

CRESYLIC ACID, a mixture of isomeric cresols and xylenols

Mine floatation wastes; sheep and cattle dips. M.M.E., 0.1 p.p.m. in hard water killed goldfish, Carassius auratus, in 5 days and 1 p.p.m. in 6-48 hours; 0.1 p.p.m. in soft water killed cladocerans Daphnia magna, in 72 hours.

CUPRIC CHLORIDE, $CuCl_2$

Powers (1917), 0.0188 p.p.m. in distilled water killed goldfish in 3 hours 30 minutes to 7 hours; Carpenter (1927), 672 p.p.m. in distilled water killed the minnow, Leuciscus phoxinus, in 82 minutes.

CUPRIC NITRATE, $Cu(NO_3)_2$

Dilling and Healey (1926), 0.0188 p.p.m. in tap water killed many tadpoles and interfered with the development of those which survived.

CUPRIC SULPHATE, $CuSO_4$. Blue vitriol.

Because of the wide use of this compound in both industry and in aquatic investigations, and because of the variation in limits of lethality copper sulphate as given by many writers, owing to differences in water, in carbonate content, and in associated substances, a larger number of references have been included for copper sulphate than for most compounds in this list. Penny and Adams (1863), 10 p.p.m. fatal to goldfish and minnows, but 5 p.p.m. under the conditions of their tests were not lethal to these fishes; Moore and Kellerman (1905), 0.143 p.p.m. in hatchery water (Cold Spring Harbor, N.Y.), maximum strength tolerated by brook trout, and 0.33 p.p.m. maximum for carp and suckers, 0.4 p.p.m. for catfish, 0.5 p.p.m. for goldfish, 1.33 p.p.m. for sunfish, 2 p.p.m. for black bass; Carpenter (1927), 399 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in 62 minutes; Catt (1934), 0.5 p.p.m. in lake water not lethal to white perch and yellow perch in 15 hours, but 1 p.p.m. in lake water killed white and yellow perch in 1 to 10 hours; M.M.E., 2 p.p.m. in hard water killed goldfish, Carassius auratus, in 24-96 hours; catfish, Ameiurus nebulosus, in 96-200 hours; 1 p.p.m., some goldfish in 72 hours; 1.25 p.p.m., the amphipods, Gammarus fasciatus and Eucrangonyx gracilis, in 17-20 hours; 10 p.p.m., the isopod, Mancasellus macrourus, in 16-48 hours; 1 p.p.m. in distilled water killed cladocerans, Daphnia magna, in 15 minutes to 2 hours, and 0.25 p.p.m. in distilled water in 30 minutes to 3 hours.

ETHYL ALCOHOL, C_2H_5OH

Fermented organic wastes, particularly fruit pulps brewery and distillery wastes. Weigelt, Saare, and Schwab (1885), a 2-hour exposure to 10,000 p.p.m. in tap water survived by tench, Tinca vulgaris, without injury; Powers (1917), 250 p.p.m. in distilled water killed goldfish in 6-11 hours.

ETHYL AMINE, $C_2H_5NH_2$

Gas wastes. Shelford (1917). 400-800 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

FERRIC CHLORIDE, $FeCl_3$

Dye industries; some ore milling operations; various chemical wastes. Powers (1917), 9 p.p.m. in distilled water killed goldfish in 20 hours; Carpenter (1927), 270 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in 90 minutes; M.M.E., 100 p.p.m. in very soft water killed goldfish, Carassius auratus, in 1 hour to 1 hour 30 minutes, but same quantity of ferric chloride in hard water was not detrimental to goldfish in a 96-hour exposure.

FERRIC POTASSIUM SULPHATE, $FeK(SO_4)_2$. Ferric alum.

Dye mordant; calico printing. Weigelt, Saare, and Schwab (1885), 1 minute exposure to 10,000 p.p.m. not fatal to tench.

FERRIC SULPHATE, $Fe_2(SO_4)_3$

Chemical industries; as a coagulant for sewage precipitation. Clark and Adams (1913), 0.716 p.p.m. in distilled water killed shiners, carp, and suckers in 12-24 hours.

FERROUS SULPHATE, $FeSO_4$. Green vitriol or copperas.

Waters from mines containing pyrites; in pickle liquor from industrial plants cleaning iron plate or wire. Weigelt, Saare, and Schwab (1885), 2,721 p.p.m. in tap water killed trout and California salmon in 31-66 minutes; Clark and Adams (1913), 2.9 p.p.m. in distilled water killed shiners, carp, and suckers in 4-24 hours; Carpenter (1927), 315 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in about 3 hours; M.M.E., 1,000 p.p.m. in hard water killed goldfish, Carassius auratus, in 2-10 hours. 100 p.p.m. in hard water apparently not harmful to goldfish in a 96-hour exposure.

GALLIC ACID, $C_6H_2(COOH)(OH)_3$

Dye wastes; tannery wastes; some chemical wastes. Penny and Adams (1863), 143 p.p.m. killed goldfish and minnows.

GLYCEROL, $(CH_2OH)_2CHOH$. Glycerine.

Soap factories. Weigelt, Saare, and Schwab (1885), tench survived 16-hour exposure at 8° C. to 100,000 p.p.m. in tap water without apparent effect.

HYDROCHLORIC ACID, HCl. Muriatic acid.

In effluents from many chemical processes.

Weigelt, Saare, and Schwab (1885), 1,000 p.p.m. in tap water caused trout to overturn helpless in 2-5 minutes; Wells (1915a), 3.6 p.p.m. in distilled water killed green sunfish, Lepomis cyanellus, in 48 hours. Standing Committee on Rivers Pollution (1924), 200 p.p.m. in distilled water produced general collapse in perch and roach; M.M.E., 166 p.p.m. in hard water killed goldfish, Carassius auratus, in 4-7 hours; 157 p.p.m. in hard water apparently did not injure goldfish in over 100 hours' exposure; 56 p.p.m. in soft water killed cladocerans, Daphnia magna, in 17-72 hours.

HYDROGEN SULPHIDE, H_2S

Produced by decomposition of many types of organic effluents, both municipal and industrial, and occurs in many trade wastes, chemical wastes, and gas wastes. This gas which readily dissolves in water is not only harmful in itself but in its decomposition may produce colloidal sulphur, which is also a pollution hazard. Weigelt, Saare, and Schwab (1885), a 3-hour exposure to 100 p.p.m. in tap water was fatal to tench, Tinca vulgaris, 8 days later, and 10 p.p.m. in tap water caused trout to float on back in 15 minutes; Shelford (1917), 4.9-5.3 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Belding (1929), 0.086 p.p.m. lethal for brook trout, Salvelinus fontinalis, 3.8 p.p.m. for the sucker, Catostomus commersonii, 4.3 p.p.m. for aquarium goldfish, Carassius auratus, and 6.3 p.p.m. for carp, Cyprinus carpio; M.M.E., 10 p.p.m. in hard water killed goldfish, Carassius auratus, in 96 hours or less, 5 p.p.m. killed some goldfish in 200 hours, and 1 p.p.m. in soft water killed cladocerans, Daphnia magna, in 72 hours or less.

IODINE, I_2

Rarely found free in stream water. Penny and Adams (1863), 28.5 p.p.m. killed minnows and goldfish.

IRON, Fe_2

See various ferric and ferrous compounds.

LACTIC ACID, $CH_3CH(OH)COOH$

A component of various dairy industries wastes. M.M.E., 654 p.p.m., in hard water killed goldfish, Carassius auratus, in 6-43 hours, but 430 p.p.m. in hard water apparently was not harmful to goldfish in exposures of over 100 hours; 170 p.p.m. in soft water killed cladocerans, Dapghnia magna, in 26-72 hours.

LEAD, Pb_2

Compounds of this metal are found in many industrial wastes and in effluents from various mining and ore milling operations. These compounds are highly toxic as has been discussed under action of heavy metals on fishes and other aquatic organisms. Two general statements concerning the lethality of lead may be given here. Other references follow under the several salts of lead. Carpenter (1927) believes that as little as 0.33 p.p.m. of lead may be lethal to fresh-water fishes, and Dilling, Healey, and Smith (1926) found that 4 p.p.m. of colloidal lead retarded the growth of young plaice.

LEAD ACETATE, $Pb(C_2H_3O_2)_2$. Sugar of lead.

Rushton (1922), 10 p.p.m. in stream water killed yearling trout; Carpenter (1925), 5 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in 4-16 hours, and 10 p.p.m. in distilled water, if renewed every other day, killed goldfish in 12 days.

LEAD NITRATE, $Pb(NO_3)_2$

Rushton (1922), 10 p.p.m. in stream water killed trout in 2 hours 15 minutes; Carpenter (1925), 250 p.p.m. in distilled water killed goldfish in 4-5 days and minnows, Leucisus phoxinus, in 2-3 hours; Dilling and Healey (1926), 1.6 p.p.m. retards growth of tadpoles and 3.3 p.p.m. lethal for tadpoles in tap water; Carpenter (1930), 165 p.p.m. in distilled water, if given sufficient exposure will kill steel-colored minnow, Notropis whipplii; common shiner, Notropis cornutus; blunt-nosed minnow, Hyborhynchus notatus; silver-mouthed minnow, Ericymba buccata; sucker-mouthed minnow, Phenacobius mirabilis; creek chug, Semotilus atromaculatus; stoneroller, Campostoma anomalum; common sucker, Catostomus commersonii; chub sucker, Eriomyzon sucetta; Johnny darter, Boleosoma nigrum; fan-tailed darter, Etheostoma flabellare; log perch, Percina caprodes; and bluegill, Lepomis pallidus; M.M.E., 100 p.p.m. in hard water fatal to goldfish, Carassius auratus, in 80 hours; 10 p.p.m. in hard water, without apparent injury to goldfish, in 96 hours' exposure.

LEAD SULPHATE, $PbSO_4$

Carpenter (1925), 25 p.p.m. in distilled water killed goldfish, Carassius auratus, in 4 days, and minnows, Leuciscus phoxinus, in 2-3 hours.

LITHIUM CHLORIDE, LiCl

Found in some mineral springs. Powers (1917), 3,750 p.p.m. in distilled water killed goldfish in 22-27 hours.

MAGNESIUM CHLORIDE, $MgCl_2$

A component of various waste waters from oil wells, and some industrial wastes. Garrey (1916), 476 p.p.m. in distilled water killed straw-colored minnow, Notropis blennius, in 4-6 days; Powers (1917), 6,757 p.p.m. in distilled water killed goldfish in from 78 hours to 21 days; Wiebe, Burr, and Faubion (1934), 5,000 p.p.m. in distilled water killed golden shiner, Notemigonus crysoleucas, in 96 hours.

MAGNESIUM NITRATE, $Mg(NO_3)_2$

Powers (1917), 12,500 p.p.m. killed goldfish in 14-16 hours.

MERCURIC CHLORIDE, $HgCl_2$. Corrosive sublimate.

Weigelt, Saare, and Schwab (1885), 500 p.p.m. in tap water killed large trout in 54 minutes; Carpenter (1927), 13.6 p.p.m. in distilled water killed minnow, Leuciscus phoxinus, in 42 minutes.

METHYL ALCOHOL, CH_3OH

A solvent in many industrial operations. Weigelt, Saare, and Schwab (1885), a 2-hour exposure to 10,000 p.p.m. in tap water was tolerated by trout without apparent injury; Powers (1917), 250 p.p.m. in distilled water killed goldfish in 11-15 hours.

METHYL MERCAPTAN, CH_3SH

A highly toxic constituent of sulphite paper pulp waste. Cole (1935a), 1 p.p.m. in lake water killed white bass, Roccus chrysops, in 1 hour, 45 minutes, or less; yellow perch (Perca flavescens), largemouth black bass (Micropterus salmoides), smallmouth black bass (Micropterus dolomieu), and bluegill (Lepomis pallidus), in 6-8 hours; and rock bass (Ambloplites rupestris), in 11 hours.

MURIATIC ACID

See hydrochloric acid.

NAPHTENIC ACIDS and naphthene derivatives.

These compounds are listed among the solutes which may occur in refinery wastes (American Petroleum Institute, 1935), and Kupzis (1902) believes the naphthenic compounds to be among the most toxic substances to fish, passing from crude oil into water. Kupzis (l.c.), using fractions containing the naphthenic acids extracted from various crude oils, gives the following lethality findings for this naphthenic acid fraction (which of course must not be considered as representing a single pure compound in this case). In tap water (hard) 3 p.p.m. killed percoid fishes, Acerina cernua, in 6-12 hours; 5 p.p.m. killed pickerel, Esox lucius, 36-48 hours; the minnow, Abramis brama, 72 hours; the red-eyed minnow, Scardinius erythrophthalmus, 26 hours; and the perch, Perca fluviatilis, in 16-23 hours; 20 p.p.m. killed carp, Cyprinus carpio, in 26-36 hours, and goldfish, Carassius auratus, in 8-16 hours. Several other European species tested are not listed here.

NAPHTHALENE, $C_{10}H_8$

Aniline and coal-tar industries. Demyanenko (1931), 10 p.p.m. lethal to fish.

NICKELOUS CHLORIDE, $NiCl_2$

Electroplating wastes; various industrial wastes. Thomas (1924), 8.1 p.p.m. in tap water killed the topminnow, Fundulus heteroclitus, in a few hours. This species, which can also live in salt water, tolerated 259.2 p.p.m. when in salt water without apparent injury during a 2-week exposure. M.M.E., 100 p.p.m. in very soft water killed goldfish, Carassius auratus, in 19-50 hours, and 10 p.p.m. in very soft water killed in 200-210 hours.

NITRIC ACID, HNO_3

Occurs in many wastes from chemical industries. It is easily broken into water and oxides of nitrogen. Weigelt, Saare, and Schwab (1885), trout after 34 minutes in 1,000 p.p.m. in tap water were helpless; Carpenter (1927), sufficient quantities to bring the water to pH 4.4 killed the minnow, Leuciscus phoxinus, in 7 hours, while quantity sufficient to bring the water to pH 5.2 was without apparent effect on this species of fish; M.M.E., 750 p.p.m. in hard water (see section on acids) killed goldfish, Carassius auratus, in 30 minutes to 1 hour, and 200 p.p.m. in hard water were without apparent effect on goldfish in exposures over 100 hours.

OCTYL ALCOHOLS, general formula $C_8H_{17}OH$

Macht and Leach (1930), 66.7 p.p.m. of primary octynol produce respiratory and neuromuscular paralysis in goldfish, Carassius auratus, in 15 minutes. The specific toxicity for fish of the individual octyl alcohols varied in the series of 23 which these observers studied, secondary octylic heptanol being among the least toxic, 200 p.p.m. producing paralysis in goldfish in 4 hours.

OXALIC ACID, $H_2C_2O_4$

Bleaching, dying, and various chemical industries. M.M.E., 1,000 p.p.m. in hard water killed goldfish, Carassius auratus, in 25-30 minutes; 200 p.p.m. in hard water produced no apparent injury during exposures of 100 hours. This acid is readily precipitated out of waters by calcium salts.

OXYGEN, O_2, and OZONE, O_3

Although the usual problem in stream pollution is to secure enough oxygen, some of the proposed treatments for organic wastes involve high oxygenation or ozonation. It seems worth while therefore to list here some tests on the effects of high oxygen and nascent oxygen. Wiebe (1933) found changes from 5.7 p.p.m. to 40.3 p.p.m. dissolved oxygen were tolerated by largemouth black bass, Micropterus salmoides, smallmouth black bass, Micropterus dolomieu, white crappie, Pomoxis sparoides, bluegill, Lepomis incisor, orange-spotted sunfish, Lepomis humilis, golden shiner, Notemigonus crysoleucas, and goldfish, Carassius auratus, for at least 24 hours. Hubbs (1930) states that minnows may be killed by 0.033 p.p.m. of nascent oxygen in the water and that fishes are irritated by ozone in quantities less than 0.01 p.p.m.

PARA-DICHLOROBENZENE, $C_6H_4Cl_2$

Demyanenko (1931), 50 p.p.m. lethal to fishes.

PHENOL, C_6H_5OH. Carbolic acid.

An important constituent of gas wastes, many chemical effluents and even some sewage products. Phenol may also come into streams from sheep dips and other establishments where livestock are disinfected. Shelford (1917), 70-75 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Powers (1917), 51 p.p.m. in distilled water killed goldfish in 1 hour 30 minutes to 2 hours 20 minutes; Demyanenko (1931), 16.6-20 p.p.m. lethal to fishes, some can live in 15 p.p.m., but their flesh acquires phenolic smell; Alexander, Southgate, and Bassindale

(1935), 0.4-0.6 p.p.m. caused trout to overturn in 8 hours 20 minutes; Adams (1927), 10 p.p.m. in Nile River water killed cladocerans, Daphina sp., and copepods, Cyclops sp.; M.M.E., 10 p.p.m. in hard water killed goldfish, Carassius auratus, in 72 hours or less, 1 p.p.m. apparently not injurious to goldfish in exposures of 100 hours; 8 p.p.m. in soft water killed cladocerans, Daphnia magna.

POTASSIUM BICARBONATE, $KHCO_3$

Penny and Adams (1863), 2,000 p.p.m. killed minnows and goldfish.

POTASSIUM CHLORIDE, KCl

Garrey (1916), 373 p.p.m. in distilled water killed straw-colored minnows, Notropis blennius, in 12-29 hours; Powers (1917), 74.6 p.p.m. in distilled water lethal for goldfish in 4 hours 40 minutes to 15 hours.

POTASSIUM CYANIDE, KCN

Ore milling operations; chemical works; electroplating; and in effluents from coke ovens. (See Tupholme, 1933.) Powers (1917), 0.78 p.p.m. in distilled water killed goldfish in 43-118 hours; Calatroni (1928), 15 p.p.m. in tap water immobilized tadpoles with fatal results; McArthur and Baillie (1929), 65 p.p.m. killed cladocerans, Daphnia magna; Southgate, Pentelow, and Bassindale (1933), 0.27 p.p.m. KCN (equal to 0.11 p.p.m. CN) at temperatures of 7° - 9° C. caused trout to float helpless on back in about 2 hours if the dissolved oxygen in the water were circa 11 p.p.m., but in 10 minutes if the water carried only 3 p.p.m. dissolved oxygen; Alexander, Southgate, and Bassindale (1935) find 0.5 p.p.m. KCN (0.2 p.p.m. CN) will cause trout to overturn and become helpless in 15 minutes; M.M.E., 1 p.p.m. in river water caused glochidia of fresh-water mussels to close, rendering them incapable of attachment on fishes, and 0.1 - 0.3 p.p.m., in hard water killed goldfish in 3-4 days.

POTASSIUM DICHROMATE, $K_2Cr_2O_7$

Ore floatation processes; chemical industries. M.M.E., 100 p.p.m. in hard water apparently not harmful to goldfish in exposures of 108 hours, 500 p.p.m. lethal to goldfish in 3 days.

POTASSIUM FERRICYANIDE, $K_3Fe(CN)_6$. Red prussiate of potash.

Penny and Adams (1863), 2,000 p.p.m. not lethal to minnows and goldfish

POTASSIUM FERROCYANIDE, $K_4Fe(CN)_6$. Yellow prussiate of potash.

Penny and Adams (1863), 2,000 p.p.m. not lethal to minnows and goldfish; Weigelt, Saare, and Schwab (1885), trout survived 1 hour exposure to 8,723 p.p.m. in tap water without symptoms.

POTASSIUM HYDROXIDE, KOH. Caustic potash.

Soap works; from some types of ashes. Wells (1915), 56 p.p.m. in distilled water killed bluegills, Lepomis pallidus, in 4 hours 25 minutes, 28 p.p.m. apparently not harmful to bluegills in 10-day exposures.

POTASSIUM NITRATE, KNO_3. Saltpeter.

Wells (1915b), 1,203 p.p.m. in tap water killed bluegills, Lepomis pallidus, in 15 days.

POTASSIUM PERMANGANATE, $KMnO_4$

This powerful oxidizing agent is frequently used to disinfect hatchery tanks and can be applied with caution to fish themselves. Adams (1927), 5 p.p.m. in Nile River water killed cladocerans, Daphnia sp., and copepods, Cyclops sp.; M.M.E., 10 p.p.m. in hard water killed goldfish, Carassius auratus, in 12-18 hours.

POTASSIUM SULPHATE, K_2SO_4

Wells (1915b), 869 p.p.m. in tap water killed bluegills, Lepomis pallidus, in 4 days.

POTASSIUM XANTHATE or potassium ethyldithiocarbonate $C_2H_5 \cdot O \cdot CS \cdot SK$

Used in ore floatation processes and on occasion to free soil from insect pests, and it is highly toxic to fishes. M.M.E., 10 p.p.m. in hard water killed goldfish in 48-96 hours, 0.1 p.p.m. in 4-5 days.

PYRETHRUM

The volatile oil in this plant product is quite toxic to fishes. Bandt (1933) 5-10 p.p.m. toxic for carp.

PYRIDINE, C_5H_5N

In gas wastes, also in waters draining recently burned-over areas where combustion near ground has been incomplete. Shelford (1917), 1,477-1,576 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour; Powers (1917), 1,869 p.p.m. in distilled water killed goldfish in 10-30 hours; Demyanenko (1931) states 1,000 p.p.m. to have a feeble effect on fishes.

QUINOLINE, $C_6H_4N : CHCH : CH$

Gas wastes. Shelford (1917), 52-56 p.p.m. and 65 p.p.m. isoquinoline in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

SAPONIN, a glucoside.

Found in various plants. Ebeling (1928) points out that wastes from potato starch factories carry sufficient saponin to be dangerous to fishes, and that consequently these wastes should be highly diluted. M.M.E., 10 p.p.m. of saponin in hard water produced marked distress in goldfish in 5 hours, 100 p.p.m. rapidly fatal, killing goldfish in 7-24 hours.

SODIUM ARSENITE, commercial preparation usually a mixture of several sodium and arsenic compounds.

Some dye and tanning processes; removal of aquatic vegetation. Since arsenic trioxide is usually dissolved in sodium hydroxide, and since each lot of commercial sodium arsenite must be assayed for its arsenic content, arsenic, arsenic trioxide, and sodium arsenite are considered under this single heading. Wiebe (1930), 7 p.p.m. of As_2O_3 in Mississippi River water was not detrimental to largemouth black bass, Micropterus salmoides, smallmouth black bass, Micropterus dolomieu, white crappie, Pomoxis sparoides, bluegill, Lepomis pallidus, golden shiner, Notemigonus crysoleucas, bullhead, Amieurus nebulosus, and goldfish, Carassius auratus, in 148-hour exposures as shown by 3 months subsequent observation. Surber and Meehan (1931), 2 p.p.m. As_2O_3 in Mississippi River water survived by the important fish food organisms, but 2.5-4 p.p.m. killed chironomid larvae, mayfly nymphs, the fresh-water shrimp, Hyalella, and odonata nymphs; the isopod Asellus sp., survived 10-21 p.p.m.; M.M.E., 1.3 p.p.m. As_2O_3 in distilled water killed Daphna magna, 8 p.p.m. hard water had no appreciable effect on the fresh-water mussel, Amblema peruviana, but 16 p.p.m. in hard water was fatal to Amblema peruviana in 3-16 days.

SODIUM CARBONATE, Na_2CO_3

Found in many chemical effluents. Clark and Adams (1913), 250-300 p.p.m. killed shiners, carp, and large suckers in tap water in a few hours; Wells (1915b), 530 p.p.m. in tap water killed bluegills, Lepomis pallidus, in 3 days.

SODIUM CHLORATE, $NaClO_3$

In some effluents from chlorine and bromine works. Not markedly toxic. M.M.E., 1,000 p.p.m. in hard water produced no apparent injuries in goldfish during exposures of 5 days duration.

SODIUM CHLORIDE, NaCl. Common Salt.

Brine works; waste waters from oil wells; effluents from some dairy industries. Garrey (1916), 2,500 p.p.m. in distilled water killed straw-colored minnow, Notropis blennius, in 9-24 days (solution, $\Delta = 0.105$); Powers (1917), 11,765 p.p.m. in distilled water killed goldfish in 17 hours; Wiebe, Burr, and Faubion (1934), 5,000 p.p.m. in distilled water killed golden shiners, Notemigonus crysoleucas, in 148 hours and largemouth black bass in 200-250 hours; M.M.E., 1 p.p.m. distilled water killed cladoceran, Daphnia magna, in 3 hours, 5,000 p.p.m. in Mississippi River water apparently not harmful to goldfish in 25-day exposure, but 10,000 p.p.m. in Mississippi River water killed goldfish in 4-10 days.

SODIUM FLUORIDE, NaF

In certain brewery and distillery wastes; also found in some soils. M.M.E., 1,000 p.p.m. in hard water killed goldfish in 60-102 hours.

SODIUM HYDROXIDE, NaOH. Caustic soda.

Soap factories; wood ashes. Clark and Adams (1913), 96 p.p.m. in tap water killed shiners, carp, and suckers in 2-10 minutes; Standing Committee on River Pollution (1924), 50 p.p.m. in distilled water was not fatal to perch and roach in a 2-hour exposure.

SODIUM NITRATE, $NaNO_3$. Chili saltpeter.

Fertilizers. Powers (1917), 1,282 p.p.m. in distilled water killed goldfish in 14 hours; M.M.E., 4,000 p.p.m. in hard water killed goldfish in 80 hours but 3,000 p.p.m. apparently did not injure goldfish in 100-hour exposures.

SODIUM SELENITE, Na_2SeO_3

Component of certain soils; pottery works. M.M.E., 100 p.p.m. in hard water killed goldfish in 8-20 hours, 10 p.p.m. in hard water killed in 98-144 hours.

SODIUM SULPHATE, Na_2SO_4. Glaubers salt.

Harukawa (1922), 500 p.p.m. in tap water not injurious to goldfish in 24 hours.

SODIUM SULPHIDE Na_2S

In some beet-sugar factory effluents and in some paper-pulp wastes. Weigelt, Saare, and Schwab (1885), a 1-hour exposure to 1,150 p.p.m. in tap water kill tench, Tinca vulgaris, 6 days later.

SODIUM SULPHITE, Na_2SO_3

Component of various paper pulp wastes and some beet sugar factory wastes. M.M.E., 100 p.p.m. in hard water killed goldfish in 96 hours.

STANNOUS CHLORIDE, $SnCl_2$

M.M.E., 1,000 p.p.m. in hard water killed goldfish in 4-5 hours.

STRONTIUM CHLORIDE, $SrCl_2$

Powers (1917), 15,384 p.p.m. in distilled water killed goldfish in 17-31 hours.

STRONTIUM NITRATE, $Sr(NO_3)_2$

Powers (1917), 9,615 p.p.m. in distilled water killed goldfish in 32-146 hours.

SUGAR, cane, $C_{12}H_{22}O_{11}$. Sucrose.

Garrey (1916), 34-218 p.p.m. in distilled water ($\Delta = 0.198$) killed straw-colored minnow, Notropis blennius, in 24-48 hours.

SULPHUR, colloidal, S_2

Lime-sulphur mixtures and dips; following decomposition of hydrogen sulphide in water. Harukawa (1922), 1,600 p.p.m. in tap water fatal to goldfish in 3 hours 30 minutes to 5 hours 15 minutes, and 2,100 p.p.m. in 48-71 minutes.

SULPHUR DIOXIDE, SO_2

Bleaching works; various chemical industries, Weigelt, Sarre, and Schwab (1885), 10 p.p.m. in tap water caused trout to float helpless in 10 minutes; Shelford (1917), 16-19 p.p.m. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

SULPHURIC ACID, H_2SO_4. Oil of vitriol.

Pickle liquor from sheet metal and wire factories; waters from coal and iron mines; various chemical wastes. Wells (1915), 7.36 p.p.m., in distilled water killed bluegills, Lepomis pallidus, in 60 hours, but 3.68 p.p.m. apparently harmless over period of 1 month. M.M.E., 59 p.p.m. in very soft water killed goldfish, Carassius auratus, in 1 hour to 1 hour 15 minutes, 138 p.p.m. in hard water in 4 hours, 100 p.p.m. in hard water apparently not injurious to goldfish in 100-hour exposures; 29 p.p.m. in soft water killed cladocerans, Daphnia magna, in 24-72 hours.

TANNIC ACID, $C_{14}H_{10}O_9$

Tannery wastes; leachings from some barks and sawmill wastes. M.M.E., 100 p.p.m. in hard water killed goldfish in 9-20 hours, but 10 p.p.m. apparently not injurious in 100-hour exposures.

TARTARIC ACID, $COOH(CHOH)_2COOH$

Dye wastes; mordant liquors; leather works; variour chemical effluents; some winery wastes. M.M.E., 1,000 p.p.m. in hard water killed goldfish in 3-4 hours, 200 p.p.m. in hard water not injurious apparently in exposures of 100 hours, 100 p.p.m. in very soft water killed goldfish in 3 hours to 3 hours 30 minutes.

THIOPHENE, C_4H_4S

Gas wastes. Shelford (1917), 27 p.p.p. in tap water killed orange-spotted sunfish, Lepomis humilis, in 1 hour.

TIN, Sn_2

See stannous chloride.

TRADE WASTES

At end of this section.

ZINC NITRATE, $Zn(NO_3)_2$

Dilling and Healey (1926) found that tadpoles survived 3-month exposure to 1.89 p.p.m. but failed to develop limb buds, that 5.7 p.p.m. killed most tadpoles and that 94.7 p.p.m. killed tadpoles quickly.

ZINC SULPHATE, $ZnSO_4$. White vitriol.

Wastes from electrolytic refineries of zinc; incrustations developing from exposed zinc sulphide ores; mine tailings; several chemical effluents. Carpenter (1927), 404 p.p.m. in distilled water killed minnows, Leuciscus phoxinus, in 3 hours 20 minutes; M.M.E., 1,000 p.p.m. in hard water killed goldfish in 1-4 hours, 100 p.p.m. fatal to many goldfish in 5 days.

TRADE WASTES

Lethal limits which will be inclusive cannot be defined for sewage and trade wastes because sewage and even such specific effluents as Steffens house waste (from certain types of beet-sugar refineries) are not constant either in composition or concentration. The effects of colloidal sulphur or ferric chloride on fishes and other aquatic animals can be determined under a variety of conditions which can be duplicated, or the toxicity of any particular sample of Steffens waste, or pickle liquor from tin-plate mills, or deazoting fluid from dye works can also be ascertained with accuracy, but to attempt to define the minimal lethal concentration or the maximal dilution for all pickle liquors from samples obtained at any plant or series of plants is both unsound scientifically and unfair to the industry. Already much confusion, often to the detriment of fisheries interests, has resulted from the misuse in this connection of various statements in the literature concerning the tolerance of fishes for a given number of p.p.m. of a particular waste, when these observations were intended to apply to a specific case.

In practical tests the writer has found that wastes taken directly from their sources in the plant, i.e., not from sewers or flumes, differ widely in toxicity to aquatic life, at different plants using the same general process. Several factors contributed to these differences. For example, the process of tanning hides with chromium compounds is basically the same in all establishments using this process, but as is well known the plant chemists in the interests of economy and efficiency frequently make minor or at times even drastic changes in the process, with, of course, resultant changes in the effluents. Variations in raw material, emergencies in plant operation, and many other conditions also call for changes in procedure which result in changes in effluent composition and concentration. There is, therefore, no standard hide vat liquor waste or pickle liquor or winery effluent, even when taken at the source, which may be used with either fairness or accuracy in the estimation of the probable hazards of another plant engaged in the same work.

Besides, as the waste moves away from its source through conduits and flumes, these differences become greater owing to the addition of cooling water, wash waters, and other effluents from different parts of the plant; so that the final mixture as poured into the stream varies not only in concentration, but also in composition as the result of the sequence in which the different effluents were mixed.

However, from the studies of various wastes before mixing, the general changes which these effluents will produce in the stream complex and the types of their actions on aquatic life can be ascertained with considerable accuracy. In Table 14 the usual effects of 29 common industrial wastes and of municipal sewage on the aquatic environment and the critical features of these actions as shown by a large series of field studies have been pointed out.

TABLE 14.—*Usual fisheries hazards of 30 common types of municipal and industrial effluents* [1]

Types of wastes	Changes in water affecting fishes						Bottom pollution blanket	Specific toxic action on fishes
	Decrease in dissolved oxygen	Hydrogen-ion concentration		Increase in specific conductance	Increase in turbidity	Increase in ammonia		
		Increase in acidity	Increase in alkalinity					
MINERAL WASTES, LITTLE BACTERIAL ACTION								
1. Erosion silt	None	None	None	None	Critical	None	Critical	None.
2. Limestone sawmills	do	do	Possible	Moderate	do	do	do	Do.
3. Asbestos works	do	do	do	do	do	do	do	Possible.
4. Mine flotation	Possible	Possible	do	do	do	do	do	Possible to critical.
5. Coal- and iron-mine drains	do	Critical	None	do	None	do	Possible	Possible.
6. Crude oil	do	None	do	None	do	do	Possible to critical.	Possible to critical.
7. Salt water from oil wells	None	do	Possible	Critical	do	do	None	Do.
ORGANIC, BACTERIAL ACTION								
8. Municipal sewage	Critical	Possible	do	Possible	Possible	Critical	Possible to critical.	Do.
9. Dairy industries	do	Critical	None	Moderate	Moderate	Moderate	do	Possible.
10. Packing plants	do	Moderate	do	do	do	Critical	Critical	Do.
11. Canning factories	do	Critical	Possible	do	do	do	Possible to critical.	Do.
12. Breweries and distilleries	do	None to moderate.	None to moderate	do	Possible	Possible	do	Do.
13. Beet sugar, pulp wastes	do	Critical	None	do	do	do	do	Possible to critcial.
14. Paper pulp	Possible to critical.	Possible	Possible	Possible	do	do	Critical	Possible.
15. Sawdust	do	do	do	do	do	do	do	Do.
CHEMICAL PROCESSES								
16. Coal-gas wastes	Possible	do	do	Moderate	None	Critical	do	Critical.
17. Spent lubricants	do	do	None	Possible	do	None	do	Possible to critical.
18. Metal refineries	None	do	Possible	do	Possible	Possible	Possible to critical.	Critical.
19. Laundries and wool washings	Moderate	None	Moderate to critical.	Moderate	Moderate	Moderate to critical.	Possible	Possible.
20. Steffens house waste	do	do	Critical	Critical	do	None	do	Critical.
21. Sulphite pulp	Moderate to critical.	Possible	Moderate to critical.	Moderate	do	Possible	Possible to critical.	Do.
22. Strawboard waste	do	None	Critical	do	do	do	do	Do.
23. Chemical works (a)	None	do	do	do	Possible	do	None	Do.
24. Chemical works (b)	Possible	Critical	None	do	None	None	do	Do.
25. Tanneries	Moderate	Possible to critical.	Possible to critical	do	Possible	Possible to critical.	Critical	Do.
26. Dye works	Possible	None to moderate.	None to moderate	do	None	None	Possible	Do.
27. Bittern liquors	None	Critical	None	Critical	do	do	None	Possible to critical.
28. Tin-plate and wire mills	None to possible.	do	do	Moderate	None to possible.	do	Possible to critical.	Do.
29. Starch factories	Possible to critical.	do	do	do	Possible	Possible	do	Do.
30. Cloth sizing	do	Possible to critical.	do	Possible	Moderate	do	do	Possible.

[1] In this table increases in both acidity and alkalinity are noted in some cases, due to the facts that two or more kinds of effluents are mixed, with one predominating at times and to changes which take place in the stream after the effluent is added.

From this table the general effects of a given effluent or comparable waste on the aquatic complex can be predicted. By field tests at the site of pollution covering the critical factors as indicated for the particular effluent (dissolved oxygen, pH, conductivity, carbonates, and other determinations for the polluted stream as the case may be), the degree of pollution can be estimated when these field data are compared with the standards given in the section on stream pollutants and aquatic environment. When specific toxic action is indicated (last section, Table 14) the limits for the expected detrimental substances can be found in the section on lethal limits.

ACKNOWLEDGMENTS

The writer is particularly indebted to Dr. H. L. Motley, assistant professor of physiology, University of Missouri; to Dr. W. A. Chipman, assistant aquatic biologist, United States Bureau of Fisheries; to Mrs. M. D. Ellis, honorary fellow in physiology, University of Missouri; to Messrs. B. A. Westfall, W. G. Davis, Paul Pierce, R. O. Jones; to Mrs. Helene Pierce Chipman; and to Misses Cornealia G. Ellis, Zella von Gremp, Nelden Bickel, and Mary McConathy, bioassayists and chemists, who through their cooperative efforts as members of the staff of the Columbia, Mo., field unit, United States Bureau of Fisheries, have carried forward during the past 4 years many analyses, assays, and experiments required to give the mass of data for these studies; to the officers and employees or the Corps of Engineers, United States Army, in various river districts, who have aided these investigations in moving U.S. Quarterboat 348, and in supplying freely river and survey data from their own work; to the many industrial concerns which have cordially opened their plants and given valuable assistance in the collection of trade waste samples; and to the University of Missouri for continued cooperation in the maintenance of the Columbia, Mo., laboratories.

BIBLIOGRAPHY

Adams, B.A. 1927. The lethal effect of various chemicals on Cyclops and Daphnia. Water Works Eng., vol. 29, pp. 361-364. New York.

Alexander, W.B., B.A. Southgate, and R. Bassindale. 1935. The survey of the River Tees. Part II, The estuary. Chemical and Biological. Technical Paper No. 5, Water Pollution Res. Bd., England. London.

American Petroleum Institute. 1935. Disposal of refinery wastes. Section III. Waste water containing solutes. Official Publication, 37 pp. New York.

American Public Health Association. 1933. Standard methods for the examination of water and sewage. Ed. 7, 180 pp. New York.

Bandt, H. 1933. Fish poisoning caused by pyrethrum. Wasser und Abwasser, Bd. 31, s. 255. fide. Chem. Absts., vol. 27, part 3, p. 5826, 1933. Easton, Pa.

Behrens, Behrend. 1925. Untersuchungen uber Aufnahme, Ausscheidung und Verteilung kleinster Bleimengen. Archiv fur experimentelle Pathologie und Pharmakologie, Bd. 109, s. 332-357 Berlin.

Belding, D.L. 1928. Toxicity experiments with fish in reference to trade waste pollution. Trans. Amer. Fish Soc., vol. 57, 1927 (1928), pp. 100-114. Washington.

Belding, D. L. 1929. The respiratory movements of fish as an indicator of toxic environment. Trans., Amer. Fish. Soc., vol. 59, pp. 238-245. Hartford, Conn.

Billiard, G. 1925. La daphnie (Daphnia pulex) consideree comme reactif biologique de la toxicite des liquides humoraux. Comptes Rendus Société de Biologie, Tome 92, pp. 1352-1354.

Birge, E.A., and C. Juday. 1911. The inland lakes of Wisconsin. The dissolved gases of the water and their biological significance. Bull., Wisconsin Geol. and Nat. Hist. Sur., no. 22, pp. 1-259. Madison, Wis.

Bond, R.M. 1933. A contribution to the study of the natural food-cycle in aquatic environments. Bull., Bingham Oceanographic Coll., vol. 4, pp. 1-89.

Brown, H.W., and M.E. Jewell. 1926. Further studies on the fishes of an acid lake. Trans., Amer. Micro. Soc., vol. 45, pp. 20-34. Menasha, Wis.

Butcher, R.W., F.T.K. Pentelow, and J.W.A. Woodley. 1927. The diurnal variation of the gaseous constituents of river waters. Parts I and II. Biochem. Jour., vol. 21, pp. 945-957, and 1423-1435. Cambridge, England.

Calatroni, R. 1928. Action du cyanure de potassium et de l'hyposulphite de sodium sûr les tetards. Comptes Rendus Société de Biologie, Tome 99, pp. 2007. Paris.

Carpenter, K.E. 1924. A study of the fauna of rivers polluted by lead mining in the Aberystwyth district of Cardiganshire. Ann., Appl. Biol., vol. 11, pp. 1-23. Cambridge, England.

Carpenter, K.E. 1925. On the biological factors involved in the destruction of river-fisheries by pollution due to lead-mining. Ann., Appl. Biol., vol. 12, pp. 1-13. Cambridge, England.

Carpenter, K. E. 1926. The lead mine as an active agent in river pollution. Ann., Appl. Biol., vol. 13, pp. 395-401. Cambridge, England.

Carpenter, K. E. 1927. The lethal action of soluble metallic salts on fishes. British Jour., Exper. Biol., vol. 4, pp. 378-390. Cambridge, England.

Carpenter, K. E. 1930. Further researches on the action of metallic salts on fishes. Jour., Exper. Zool., vol. 56, pp. 407-422. Philadelphia.

Catt, J. 1934. Copper sulphate in the elimination of coarse fish. Trans., Amer. Fish. Soc., vol. 64, pp. 276-279. Hartford, Conn.

Chipman, W. A. 1934. A new culture medium for cladocerans. Science, vol. 79. pp. 59-60. New York.

Clark, H.W., and G.O. Adams. 1913. Studies of fish life and water pollution. Forty-fourth Annual Report of the State Board of Health of Massachusetts, 1912 (1913), Pub. Doc. No. 34, Massachusetts State Board of Health, vol. 6, pp. 336-345. Boston.

Cole, A.E. 1935a. The toxicity of methyl mercaptan for freshwater fish. Jour. Pharm. and Exper. Ther., vol. 54, pp. 448-453. Baltimore.

Cole, A.E. 1935b. Water pollution studies in Wisconsin. Effects of industrial (pulp and paper mill) wastes on fish. Sewage Works Jour., vol. 7, pp. 280-302.

Coventry, F.L., V.E. Shelford, and L.F. Miller. 1935. The conditioning of a chloramine treated water supply for biological purposes. Ecology, vol. 16, pp. 60-66. Brooklyn, N.Y.

Creaser, C.W. 1930. Relative importance of hydrogen-ion concentration, temperature, dissolved oxygen, and carbon dioxide tension, on habitat selection by brook trout. Ecology, vol. 11, pp. 246-262. Lancaster, Pa.

Davis, H.W. 1934. Discussion. Trans. Amer. Fish. Soc., vol. 64, pp. 280. Hartford, Conn.

Demyanenko, V. 1931. Poisoning of fish by waste waters from chemical factories and the fish test. (In Russian). *Fide*. Chem. Abst., vol. 27, part 2, p. 2746, 1933. Easton, Pa.

Dilling, W.J., and C.W. Healey. 1926. Influence of lead and the metallic ions of copper, zinc, thorium, beryllium, and thallium on the germination of frogs' spawn on the growth of tadpoles. Ann., Appl. Biol., vol. 13, pp. 177-188. Cambridge, England.

Dilling, W.J., C.W. Healey, and W.C. Smith. 1926. Experiments on the effects of lead on the growth of plaice (*Pleuronecies platessa*). Ann., Appl. Biol., vol. 13, pp. 168-176. Cambridge, England.

Domogalla, B.P., C. Juday, and W.H. Peterson. 1925. Forms of nitrogen found in certain lake waters. Jour., Biol. Chem., vol. 63, pp. 269-285. Baltimore.

Ebeling, G. 1928. Mittheilungen des Fisherei-Vereins, Bd. 32, s. 521-522. Berlin. *Fide*. Chem. Abs., vol. 23, p. 3042, 1929. Easton, Pa.

Ellis, M.M. 1919. Respiratory volumes of men during short exposures to constant low oxygen tensions attained by rebreathing. Amer. Jour. Phys., vol. 50, pp. 267-279. Baltimore.

Ellis, M.M. 1932. Pollution of the Coeur d'Alene River and adjacent waters by the mine wastes. Manuscript report to the Commissioner, U.S. Bur. Fish., 132 pp. Washington.

Ellis, M.M. 1934. A photoelectric apparatus for turbidity and light penetration measurement. Science, vol. 80, pp. 37-38. New York.

Ellis, M.M. 1935a. Water purity standards for fresh water fishes. Special Report, U. S. Bur. Fish., pp. 1-14. Washington.

Ellis, M.M. 1935b. A study of the Mississippi River from Chain of Rocks, St. Louis, to Cairo, Ill., with special reference to the proposed introduction of ground garbage into the river by the city of St. Louis. Manuscript report to the Commissioner, U.S. Bur. Fish., 37 pp. Washington.

Ellis, M.M. 1936a. Erosion silt as a factor in aquatic environments. Ecology, vol. 17, pp. 29-42. Lancaster. Pa.

Ellis, M.M. 1936b. The effects of pollution on fish. *In press.* Proceedings of North American Wild Life Conference, February, 1936. Washington.

Ellis, M.M., and W.A. Chipman. 1936. Studies of the toxicity of ammonium compounds for aquatic animals. *In* MS.

Engineering. 1935. The sewage treatment system of the city of Munich. Engineering, vol. 140, pp. 679-682. London.

Gardiner, J.A., and G. King. 1922. Respiratory exchange in fresh-water fish. Part IV. Further comparison of goldfish and trout. Biochem., Jour., vol. 16, pp. 729-735. Cambridge, England.

Gardiner, J.A., G. King, and E.B. Powers. 1922. The respiratory exchange in fresh-water fish. Part III. Goldfish. Biochem. Jour., vol. 16, pp. 523-529. Cambridge, England.

Garrey, W.C. 1916. The resistance of fresh water fish to changes of osmotic and chemical conditions. Amer. Jour. Phys., vol. 39, pp. 313-329. Baltimore.

Gillespie, L.J. 1920. Colorimetric determination of hydrogen-ion concentration without buffer mixtures, with especial reference to soils. Soil Science, vol. 9, pp. 115-136. Baltimore.

Harukawa, C. 1922. Preliminary report on toxicity of colloidal sulphur to fish. Trans., Amer. Fish. Soc., vol. 52, pp. 219-224. Washington.

Hodgman, C.D. 1935. Handbook of Chemistry and Physics. Ed. 20, 1951 pp. Chemical Rubber Publishing Co. Cleveland, Ohio.

Hubbs, C.L. 1930. The high toxicity of nascent oxygen. Phys. Zool., vol. 3, pp. 441-460. Chicago.

Juday, C., E.B. Fred, and F.C. Wilson. 1924. The hydrogen-ion concentration of certain Wisconsin lakes waters. Trans. Amer. Micro. Soc., vol. 43, pp. 177-190. Menasha, Wis.

Kemmerer, G., J.F. Bovard, and W.R. Boorman. 1923. Northwestern lakes of the United States: Biological and chemical studies with reference to possibilities in production of fish. Bull., U.S. Bur. Fish., vol. 39, 1923-24, pp. 51-140. Washington.

Keys, A.B. 1930. Influence on varying oxygen tension upon the rate of oxygen consumption of fishes. Bull., Scripps Inst. Ocean., Technical Series, vol. 2, pp. 307-317. La Jolla, Calif.

Keys, A.B., and E.N. Willmer. 1932. Chloride secreting cells in the gills of fishes, with special reference to the common eel. Jour. Phys., vol. 76, pp. 368-378. Cambridge, England.

Kolff, W.M. 1908. Untersuchungen uber die Herztatigkeit bei Teleostiern. Archiv fur die gesammte Physiologie des Menschen und der Tiere, Bd. 122, s. 37-97. Berlin.

Kupzis, J. 1901. Ueber dem niedrigsten fur das Leben der Fische northwendigen Sauerstoffgehalt des Wassers und uber die fur dieselben giftigen Mengen im Wasser geloster Kohlensaure. Zeitscrift fur Untersuchen der Nahrungs - und Genussmittel, sowie der Gebrauchs - gegenstande, Bd. 4, s. 631-368. Berlin.

Kupzis, J. 1902. Die Naphthafischgifte und ihr Einfluss auf Fische, andere Tiere und Bakterien. Zeitschrift fur Fischerei und deren Hilfswissenschaften, Bd. 9, s. 144-167. Berlin.

Macht, D.I., and H.P. Leach. 1930. Pharmacological studies of twenty-three isomeric octyl alcohols. Jour. Pharm. and Exper. Thera., vol. 39, pp. 71-79. Baltimore.

Marsh, M.C. 1907. The effect of some industrial wastes on fishes. Water-Supply and Irrigation Paper No. 192, U. S. Geol. Sur., pp. 337-348. Washington.

Marsson, M. 1911. The significance of flora and fauna in maintaining the purity of natural waters, and how they are affected by domestic sewage and industrial wastes. (English translation by E. Kuichling.) Engineering News, vol. 66, pp. 246-250. New York.

Massachusetts State Board of Health. 1913. Forty-fourth Annual Report of the State Board of Health of Massachusetts, 1912 (1913). Public Document No. 34, Massachusetts State Board of Health, vol. 6, pp. 1-779. Boston.

McArthur, M.C., and W.H.T. Baillie. 1929. Metabolic rates and their relation to longevity in *Daphnia magna*. Jour. Exper. Zool., vol. 53, pp. 243-268. Philadelphia.

McCay, C.M., and H.M. Vars. 1931. Studies upon fish blood and its relation to water pollution. *In* Biological survey of the St. Lawrence Watershed, Supplement to Twentieth Annual Report of the New York Conservation Department, pp. 230-233. Albany, N.Y.

McDonald, M. 1885. Effect of waste products from Page's Ammoniacal Works upon young shad fry. Bull., U.S. Fish. Com., vol. 5, pp. 313-314. Washington.

Moore, G.T., and K.F. Kellerman. 1905. Copper as an algicide and disinfectant in water supplies. Bur. Plant Ind., Bull. No. 76, 55 pp. Washington.

Munch, J. C. 1931. Bioassays, a handbook of quantitative pharmacology, 958 pp. Williams & Wilkins Co. Baltimore.

Paton, D.N. 1904. Observations on the amount of dissolved oxygen in water required by young salmonidae. Proc. Royal Soc. Edinburg, vol. 24, pp. 145-150. Edinburgh.

Pearsall, W.H. 1930. Phytoplankton in the English Lakes. I. The proportion in the waters of some dissolved substances of biological importance. Jour. Ecology, vol. 18, pp. 306-320. London.

Penny, C., and C. Adams. 1863. Fourth Report, Royal Commission on Pollution of Rivers in Scotland, vol. 2, Evidence, pp. 377-391. London.

Plehn, M. 1924. Praktikum der Fischkrankheiten. In Handbuch der Binnenfischerei Mitteleuropas, Bd. I, s. 301-470. Stuttgart.

Powers, E.B. 1917. The goldfish (*Carassius carassius*) as a test animal in the study of toxicity. Illinois Biol. Mono., vol. 4, pp. 127-193. Urbana, Ill.

Powers, E.B. 1921. Experiments and observations on the behavior of marine fishes towards the hydrogen-ion concentration of the sea water, in relation to their migratory movements and habitat. Publications, Puget Sound Biol. Sta., vol. 3, 1921-25, (1925), pp. 1-22. Seattle, Wash.

Powers, E.B. 1922. The physiology of the respiration of fishes in relation to the hydrogen-ion concentration of the medium. Jour. Gen. Phys., vol. 4, pp. 305-317. New York.

Powers, E.B. 1929. Fresh water studies: The relative temperature, oxygen content, alkali reserve, the apparent carbon dioxide tension and the pH of the waters of certain mountain streams at different altitudes in Smoky Mountain National Park. Ecology, vol. 10, pp. 97-111. Lancaster, Pa.

Powers, E. B. 1930. The relation between pH and aquatic animals. Amer. Nat., vol. 64, pp. 342-366. Boston.

Powers, E. B. 1932. The relation of respiration of fishes to environment. Ecol. Mono., vol. 2, pp. 385-473. Durham, N.C.

Richardson, R.E. 1928. The bottom fauna of the middle Illinois River, 1913-1925; its distribution, abundance, valuation, and index value in the study of stream pollution. Bull., Nat. Hist. Sur., State of Illinois, vol. 17, pp. 387-475. Urbana, Ill.

Rushton, W. 1922. Biological notes. Salmon and Trout Magazine. London.

Ruttner, F. 1926. Bermerkungen uber Sauerstoffgehalt der Gewasser und dessen respiratorischen Wert. Naturwissenschaften, Bd. 14, s. 1237-1239. Berlin.

Shelford, V.E. 1917. An experimental study of the effects of gas waste upon fishes, with especial reference to stream pollution. Bull., Illinois State Lab. of Nat. Hist., vol. 11, pp. 381-412. Urbana, Ill.

Shelford, V.E. 1929. Laboratory and Field Ecology. Williams & Wilkins Co., 608 pp. Baltimore.

Smith, H.W. 1929. The excretion of ammonia and urea by the gills of fish. Jour. Biol. Chem., vol. 81, pp. 727-742. Baltimore.

Smith, H.W. 1930. The absorption and excretion of water and salts by marine Teleosts. Amer. Jour. Phys., vol. 93, pp. 480-505. Baltimore.

Southgate, B.A., F.T.K. Pentelow, and R. Bassindale. 1933. The toxicity to trout of potassium cyanide and p-cresol in water containing different concentrations of dissolved oxygen. Biochem. Jour., vol. 27, pp. 983-985. Cambridge, England.

Standing Committee on Rivers Pollution. 1924. River Pollution and Fisheries. Min. Agri. and Fish., 42 pp. London.

Steinmann, P. 1928. Toxikologie der Fische. Handbuch der Binnenfischerei Mitteleuropas, Bd. 6, s. 289-342. Stuttgart.

Surber, E.W. 1936. The culture of daphnia. The Progressive Fish Culturist, U.S. Bur. Fish., no. 17, pp. 1-6. Washington.

Surber, E.W., and O.L. Meehan. 1931. Lethal concentrations of arsenic for certain aquatic organisms. Trans., Amer. Fish. Soc., vol. 61, pp. 225-239. Hartford, Conn.

Suter, R., and E. Moore. 1922. Stream pollution studies. Bull. New York State Conser. Com., pp. 3-27. Albany, N.Y.

Thomas, A. 1924. Studies on the absorption of metallic salts by fish in their natural habitat. II. The absorption of nickel by *Fundulus heteroclitus*. Jour. Biol. Chem., vol. 58, pp. 671-674.

Thompson, D.H. 1925. Some observations on the oxygen requirements of fishes in the Illinois River. Bull., Nat. Hist. Sur., Vol. 15, pp. 423-437. Urbana.

Tupholme, C.H.S. 1933. Death of fish from cyanides in coke-oven effluents. Indus. and Eng. Chem., News Ed., vol. 11, p. 211. Easton, Pa.

Turner, C.L. 1927. Biological survey of Fox, Wisconsin, and Flambeau Rivers, Wisconsin, with special reference to pollution. *In* Stream Pollution in Wisconsin, Special Report of the Conservation Commission and State Board of Health of Wisconsin, pp. 242-276. Madison.

Weigelt, C., with O. Saare and L. Schwab. 1885. Die Schadigung von Fischerei and Fischzucht durch Industrie und Haus Abwasser. Archiv fur Hygiene, Bd. 3, s. 39-117. Munchen und Leipzig.

Welch, P.S. 1935. Limnology. McGraw-Hill Co., 471 pp. New York and London.

Wells, M.M. 1913. The resistance of fishes to different concentrations and combinations of carbon dioxide and oxygen. Biol. Bull., vol. 25, pp. 323-347. Lancaster, Pa.

Wells, M.M. 1915a. Reactions and resistance of fishes in their natural environment to acidity, alkalinity and neutrality. Biol. Bull., vol. 29, pp. 221-257. Lancaster, Pa.

Wells, M.M. 1915b. The reactions and resistance of fishes in their natural environment to salts. Jour. Exper. Zoo., vol. 19, pp. 243-283. Philadelphia.

Wells, M.M. 1918. The reactions and resistance of fishes to carbon dioxide and carbon monoxide. Bull., Illinois State Lab. Nat. Hist., vol. 11, pp. 557-578. Urbana.

West Riding River Board, Laboratory Staff of. 1930. Biological survey of the River Wharfe. I. Dissolved substances of biological interest in the waters. Jour. of Ecol., vol. 18, pp. 274-285. London.

Wiebe, A.H. 1928. Biological survey of the upper Mississippi River with special reference to pollution. Bull., Bur. Fish., vol. 43, pp. 137-167. Washington.

Wiebe, A.H. 1930. Notes on the exposure of young fish to varying concentrations of arsenic. Trans., Amer. Fish. Soc., vol. 60, pp. 270-276. Hartford, Conn.

Wiebe, A.H. 1931a. Notes on the exposure of several species of pond fishes to sudden changes in pH. Trans., Amer. Micro. Soc., vol. 50, pp. 380-393. Menasha, Wis.

Wiebe, A.H. 1931b. Dissolved phosphorus and inorganic nitrogen in the water of the Mississippi River. Science, vol. 73, p. 652. Lancaster, Pa.

Wiebe, A.H. 1933. The effect of high concentrations of dissolved oxygen on several species of pond fishes. Ohio Jour. of Science, vol. 23, pp. 110-126. Columbus, Ohio.

Wiebe, A.H., J.G. Burr, and H.E. Faubion. 1934. The problem of stream pollution in Texas with special reference to salt water from oil fields. Trans., Amer. Fish. Soc., vol. 64, pp. 81-85. Hartford, Conn.

Winslow, C.E.A., and E.B. Phelps. 1906. Investigations on the purification of Boston Sewage. U.S. Geol. Sur., Water Supply and Irrigation Papers No. 185, 163 pp. Washington.

Wisconsin State Board of Health. 1927. Stream Pollution in Wisconsin, Special Report, a joint report of the Conservation Commission and the State Board of Health in Wisconsin, concerning activities in the control of stream pollution, from July 1, 1925, to December 31, 1926, 328 pp. Madison.

Reproduced With Permission From:
TRANSACTIONS OF THE AMERICAN FISHERIES SOCIETY
82(1953) : 156-165

THE EFFECTS OF SEWAGE POLLUTION ON THE FISH POPULATION OF A MIDWESTERN STREAM

Max Katz and Arden R. Gaufin

Biology Section, Environmental Health Center
Public Health Service, Cincinnati, Ohio

ABSTRACT

Collections of fish were made in Lytle Creek, Clinton County, Ohio, to determine the effect of the partially treated domestic sewage of Wilmington, Ohio, on the fish populations of this stream. The farther the collecting localities were downstream from the plant effluent, the greater were the numbers of species and individuals found. In the septic areas, immediately below the effluent, no fish were observed or collected. Two miles below, in the recovery zone, an average of 1.2 species and 2.2 individuals were taken per collection, with 8 different species represented. Three miles below, also in the recovery zone, 12 species were represented in our collections, with a mean of 1.7 species and 10.8 individuals per collection. In the lower portion of the recovery zone, 4.4 miles below the effluent, 32 species were taken, with a mean of 12.7 species and 197 individuals. In the clean-water zone, 36 species were taken, with a mean of 16.7 species and 294 individuals.

No species could be used as indicators of pollution although several were relatively tolerant of unfavorable conditions. The various species of darters and black bass were highly sensitive and their presence was usually indicative of favorable conditions.

During the winter when the stream flow was at a maximum and the dissolved oxygen was adequate in all sections of the stream, the fish failed to move into the areas they found intolerable during the summer periods of low flow.

INTRODUCTION

Field studies have been conducted for the past 2-1/2 years on Lytle Creek, a small Ohio stream polluted with domestic sewage. These investigations have been directed toward an understanding of the faunal and environmental changes in streams caused by organic pollution and of the resulting damage to aquatic populations. The effects of domestic sewage on some physical and chemical characteristics of the stream and upon the composition of the invertebrate fauna have been discussed by Gaufin and Tarzwell (1952). This paper will treat in particular the effects of sewage pollution upon the species composition, relative abundance, and distribution of the fishes of Lytle Creek.

DESCRIPTION OF LYTLE CREEK

Lytle Creek lies about 45 miles northeast of Cincinnati, in Clinton County, Ohio, and flows into Todd's Fork, which is part of the Little Miami River system. It is a permanent stream, approximately 11 miles long, 3 to 35 feet wide during low-water stages, and drains a watershed of about 27 square miles. The depth of the stream varies from a few inches in the riffles to more than 6 feet in a few pools. The average gradient is 25 feet per mile. The base level of Lytle Creek lies within a meander belt of alluvial origin, the Genessee silt loam. The stream runs through Wisconsin glacial till, except for a short section located about 3 miles from the mouth, where it cuts down to Ordovician limestone.

The principal sources of flow are drainage from adjacent agricultural areas and the effluent of the Wilmington Sewage Treatment Plant, which enters the stream about 7 miles above its mouth. The Sewage Treatment Plant serves the 7,000 inhabitants of Wilmington, Ohio, a non-industrial community. The sewage is treated by a chemical precipitation process, which removes most of the solids and reduces the biochemical oxygen demand of the wastes by about 50 percent. By the time the partially treated wastes are discharged to the stream, decomposition is well advanced, and little or no dissolved oxygen is present in the plant effluent. During normal low flows, the effluent comprises 80 to 90 percent of the total stream volume, causing septic conditions immediately below the outlet.

Lytle Creek is deemed particularly favorable for study because it has only one major source of pollution, is without permanent tributaries below the sewage outfall, and exhibits a wide range of pollutional conditions in its septic, recover, and clean-water zones.

Six stations representing the different polluted and clean-water zones were selected for routine sampling. Their distances in miles from the mouth of the streams were as follows: upper clean-water zone, 8.7; zone of intermittent pollution, 7.6; septic zone, 5.2; recovery zone, 4.2 and 2.8; and clean-water zone, 1.0. The location of the sewage treatment plant outlet was 7.2 miles from the stream outlet.

METHODS

Fish collections were made monthly from May 1950 until July 1951, with the exception of December 1950 and January 1951, when ice cover or flood conditions made seining impossible. Collections were resumed in February 1952 and continued until July of that year.

Samples were taken with two nets, each of 1/4-inch-square mesh: one, a straight seine 15 feet long and 5 feet deep; the second, a bag seine 35 feet long and 6 feet deep. At each station, collections were made in the pools and rifles in order to insure capture of species with different habitat preferences. It was possible to sample all stations in one day. Approximately the same amount of time was spent at each station in order to equalize fishing effort at the various sampling points. However, fish were so few at Stations 5.2 and 4.2 that a longer period was required to establish the presence or absence of fish at those localities. Some of the spring and fall collections were, of necessity, made at periods of relatively high water, during which it was impossible to sample completely some of the deeper areas. The collected fish were preserved and later identified in the laboratory.

At best, the collection methods were only roughly quantitative, and the data obtained are not believed, therefore, to be amenable to rigorous statistical treatment. Yet, as will be seen in later sections of this paper, the observed differences in the fish populations at the various stations were so marked that sampling errors are deemed relatively unimportant, and there can be little doubt of the validity of the major conclusions derived from this study.

Information on the diurnal and seasonal variations of some of the chemical and physical characteristics of Lytle Creek was obtained. Several 24-hour series of observations were made at the various stations during which dissolved oxygen, pH, and temperatures were determined hourly. These data are summarized in Table 1.

A sharp-crested, rectangular, contracted wier and a float-level recorder were placed in the stream about 3 miles above its mouth for the continuous recording of stream flow. During the winter and spring, flows averaged about 6 to 7 cubic feet per second except during frequent floods of 40 cubic feet per second or more. In the late summer and fall, the flow dropped to 1 cubic foot per second.

ABUNDANCE OF FISH BY SPECIES AND NUMBER AT THE DIFFERENT STATIONS

The 20 monthly collections yielded a total of 11,235 fish belonging to 6 families and 38 species (Table 2). Not all of these species were found at all times of the year, nor were all species found at any one station.

Not only was the species composition markedly different at the various stations, but the numbers of individuals taken varied also. Fourteen species were taken at Station 8.7, a clean-water area well upstream from the treatment-plant outlet. On the average, 6.5 species and 57 individuals were taken there per monthly collection, with minnows and darters predominant. At Station 7.6, which is a short distance above the treatment plant, 9 different species were collected; the mean number of species and individuals taken per trip were 3.9 and 30, respectively. Various minnows and the white sucker were most abundant. It is suspected that the population in this area is adversely affected by street drainage from Wilmington and periodic leakage of raw sewage from siphons transferring wastes under the stream. This slight pollution, may have repelled some of the more sensitive species resident at Station 8.7.

No fish were ever taken or observed at the sewage outlet, Station 7.2, or at Station 6.5, in the septic zone, although several species of Diptera and other tolerant insects were found to be abundant there by Gaufin and Tarzwell (1952). In the recovery zone, at Station 5.2, the fish fauna was limited, for on the average only 1.2 species and 2.2 individuals were taken. Although 8 different species were collected here, the occurrence of fish was sporadic, and fish were taken in only 8 of 20 collections. The predominant fishes were the creek chub and the fathead minnow.

At Station 4.2, conditions apparently were still marginal for fish, although the stream bottom and the water often looked relatively clean, indicating that much purification had taken place in the stream mile intervening between Stations 5.2 and 4.2. Here, only 1.7 species and 10.8 individuals were collected per trip and fish were caught in 12 of 20 collections. A total of 12 different species were found at this station with the creek chub, the blacknose dace, and the fathead minnow the most abundant.

In the next 1.4 miles, stream conditions improved greatly. Fish were taken in all collections at Station 2.8. A total of 32 species were observed; the average catch contained 12.7 species and 197 individuals. At Station 1.0, where the stream had recovered from its pollutional load, 36 species were captured and the average collection yielded 16.7 species and 294 individuals.

POLLUTION AND FISH DISTRIBUTION

As indicated in Figure 1 and Table 3, the species and numbers of fishes increased progressively with distance downstream from the sewage-plant outfall. At Stations 5.2 and 4.2, which are in the upper portion of the recovery zone, fish were frequently absent or were represented by very few species and individuals, whereas in the lower zone of recovery and the clean-water zone, at Stations 2.8 and 1.0, large numbers of species and individuals were present at all times.

The species composition of the collections was variable even in the clean-water zone. On two occasions, March 1951 and February 1952, 21 species were taken at Station 1.0, whereas only 8 species were collected there in July 1950. There is insufficient evidence for judging whether the difference in the numbers of species taken at different times at any station are related to the migratory habits of the fish or to failure to capture all the species present. It is known that several species of suckers undertake spawning migrations from Todd's Fork into Lytle Creek. The pollution of Lytle Creek has rendered unsuitable large spawning areas of the stream which undoubtedly were formerly

TABLE 1.—*The maximum and minimum dissolved-oxygen content (p.p.m.) and water temperature (°F.) at stations in recovery and clean water zones of Lytle Creek*

Station[1]	Item		Dates of hourly sampling over 24-hour period								
			April 24 1951	May 1 1951	May 9, 10 1951	June 28, 29 1951	August 15 1951	August 22, 23 1951	February 22, 23 1951	June 19, 20 1952	July 22, 23 1952
5.2	Temperature	Maximum......	60.5	76	70	80.5	80	75	41	....	....
		Minimum......	54	65	59	77	71	61	34	....	....
	Oxygen	Maximum......	12.4	19.1	19.4	11.6	7.3	6.9	11.9	....	....
		Minimum......	5.4	0.8	2.9	0.2	0.0	0.2	8.2	....	....
4.2	Temperature	Maximum......	66	76	....	....	77	71.5	41	77	82
		Minimum......	54	65	....	....	72	55	34	67	71
	Oxygen	Maximum......	16.4	18.1	....	....	5.4	5.5	12.5	3.0	3.0
		Minimum......	6.5	1.0	....	....	1.4	2.0	8.6	0.6	0.7
2.8	Temperature	Maximum......	69	79	....	87	87	80	42	86	91
		Minimum......	54	65	....	72	72	59	32	64	70
	Oxygen	Maximum......	13.8	14.3	....	10.1	12.3	14.7	13.9	11.1	10.2
		Minimum......	8.1	1.6	....	3.6	1.6	3.2	10.9	3.8	3.1
1.0	Temperature	Maximum......	70	....	70	....	....	73	41	....	....
		Minimum......	54	....	58	....	....	65.5	32	67	....
	Oxygen	Maximum......	13.5	....	14.2	....	....	9.6	13.8	....	....
		Minimum......	6.9	....	5.2	....	....	5.3	10.7	3.8	3.7

[1] Miles above mouth of stream

TABLE 2.—*Fish species taken at selected stations in Lytle Creek, Clinton County, Ohio, and their relative abundance*

["x" indicates presence of species at station. Superscript numerals indicate relative abundance of species]

Species	Common name	Station[1]					
		8.2	7.6	5.2	4.2	2.8	1.0
Carpiodes cyprinus	Quillback carpsucker	..	..	..	..	x	x
Catostomus c. commersoni	White sucker	x	x^3	x	x^5	x^5	x^{10}
Moxostoma erythrurum	Golden redhorse	..	..	..	..	x	x^8
Hypentelium nigricans	Hog sucker	..	..	..	..	x	x
Campostoma a. anomalum	Stoneroller	x	x	..	x	x^3	x^5
Rhinichthys atratulus	Blacknose dace	x	..	..	x^2	x^{10}	x
Semotilus a. atromaculatus	Creek chub	x^2	x^1	x^3	x^1	x^1	x^3
Pimephales p. promelas	Fathead minnow	x^5	x^2	x^1	x^2	x	x
Hyborhynchus notatus	Bluntnose minnow	x^3	x^4	x	x	x^4	x^1
Phenacobius mirabilis	Suckermouth minnow	..	..	..	..	x	x
Hybopsis a. amblops	Northern bigeye chub	..	..	..	..	x	x
Ericymba buccata	Silverjaw minnow	x	..	..	x	x^2	x^6
Notropis cornutus	Common shiner	..	..	x	x	x^6	x^5
Notropis ardens	Rosefin shiner	..	..	..	..	..	x
Notropis rubellus	Rosyface shiner	..	..	..	..	x	x
Notropis photogenis	Silver shiner	..	..	..	..	x^8	x^7
Notropis deliciosus	Sand shiner	x	..	x	x^4	x	x^4
Notropis spilopterus	Spotfin shiner	..	..	..	..	x	x
Notropis volucellus	Mimic shiner	x	..	..	..	x^7	x^9
Notemigonus crysoleucas	Golden shiner	..	..	..	..	x	..
Ameiurus m. melas	Black bullhead	..	..	x^4	x	x^9	x
Ameiurus n. nebulosus	Brown bullhead	..	..	..	..	x	x
Ameiurus natalis	Yellow bullhead	..	..	..	..	x	x
Poecilichthys flabellaris	Barred fantail	..	..	..	..	..	x
Poecilichthys spectabilis	Orange throated darter	x^1	x	..	x	..	x
Poecilichthys caeruleus	Rainbow darter	..	..	..	..	x	x
Etheostoma b. blennioides	Greensided darter	x	..	..	..	x	x
Boleosoma n. nigrum	Johnny darter	x^4	x	..	..	x	x
Percina caprodes	Logperch	..	..	..	..	..	x
Stizostedion v. vitreum	Walleye	..	..	..	..	..	x
Micropterus dolomieui	Smallmouth bass	..	..	..	..	x	x
Micropterus punctulatus	Spotted bass	..	..	..	..	..	x
Micropterus salmoides	Largemouth bass	..	..	..	..	x	..
Lepomis cyanellus	Green sunfish	x	x^4	x^2	x	x	x
Lepomis macrochirus	Bluegill	x	x	..	..	x	x
Ambloplites rupestris	Rock bass	..	..	..	..	x	x
Pomoxis annularis	White crappie	..	..	..	..	x	x
Percopsis omiscomaycus	Trout-perch	..	..	..	..	x	x

[1] Miles above mouth of stream

used by these migratory species, and it has, therefore, an adverse although indirect effect on the Todd's Fork fish populations.

Although Station 8.7 is unpolluted, only 14 species of fish were captured there during the collection period. This figure is less than half the number taken at the lower clean-water station, 1.0. It is recognized (Thompson and Hunt, 1930) that the number of species found in the headwaters of a small stream usually is less than that found in the downstream areas of the same stream. It is possible, however, that the relative scarcity of fish species at Station 8.7 is due in part to the pollutional barrier that prevents fish from moving upstream.

Although certain fishes in Lytle Creek seem to be more tolerant of organic pollution than others, no single species shows a preference for polluted water. Even the tolerant headwater species sometimes found at Stations 5.2 and 4.2 were captured only in small numbers. The creek chub was the most common species in the polluted zones and was often found when no other species were present at these stations. Yet the creek chub was common elsewhere in Lytle Creek and was often predominant even in the downstream cleaner-water zones. Neither the creek chub nor any other tolerant species can, therefore, be regarded as a pollution indicator in the usual sense of the term. The number of species present and their relative abundance are the most important considerations when pollutional conditions in a stream are being evaluated.

No single species, therefore, should be designated as a positive pollution indicator. When careful collection yields only a small number of species which are known to be exceptionally tolerant of pollution, then one may be justified in concluding that the stream station under investigation is a poor habitat for fish. Before that conclusion can be drawn, however, other collections must be made in sections of the stream which are known to be unpolluted, or in adjacent unpolluted streams of the same character, for the purpose of determining the normal population. It is important furthermore, to determine which species are sensitive and are among the first to disappear from waters subject to pollution. In Lytle Creek the various species of darters and black bass seem to fall into this group. Gerking (1947) and Jackson and Henderson (1942) also noted the rapid disappearance of darters in the presence of mixed wastes. In Lytle Creek, at Station 8.7, a clean-water station above the sewage outlet, darters comprised 38 percent of the population; at Station 7.6, where pollution is relatively

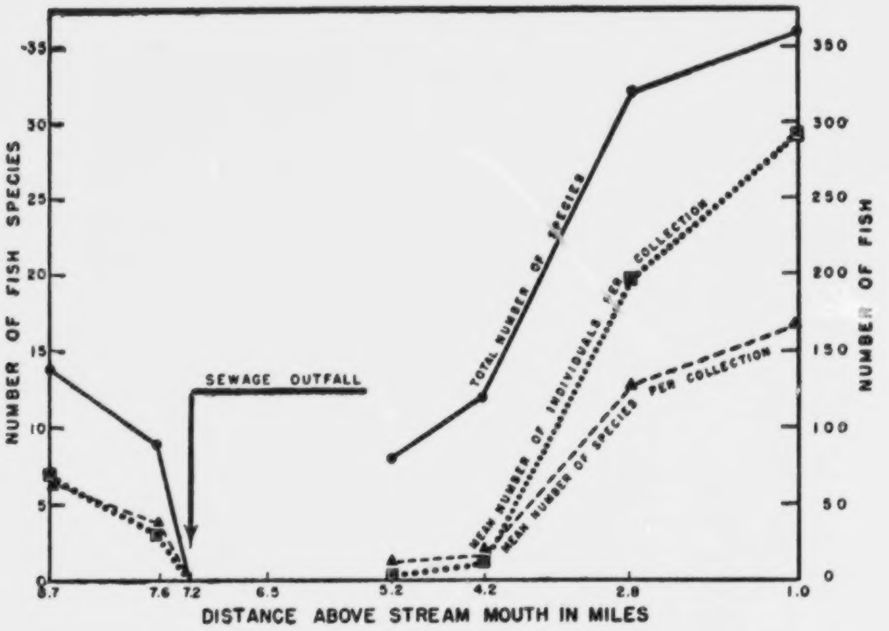


Figure 1 - Total number of fish species, and the mean number of individuals and species taken per collection at different stations in Lytle Creek.

TABLE 3.—*Number of fish species and individuals taken at the various stations on Lytle Creek*

Item	Station[1]						
	8.2	7.6	Sewage outlet	5.2	4.2	2.8	1.0
Total number of species	14	9	0	8	12	32	36
Range in number of species per collection	4-10	2-8	.	0-5	0-9	8-18	8-23
Mean number of species per collection	6.5	3.9	..	1.2	1.7	12.7	16.7
Total number of individuals	739	420	0	44	216	3,938	5,878
Range in numbers of individuals per collection	19-104	2-146	..	0-10	0-50	23-553	58-572
Mean number of individuals per collection	57	30	..	2.2	10.8	197	294
Number of collections	13	14	..	20	20	[2]21	[2]21

[1] Miles above mouth of stream; location of sewage outlet was 7.2 miles.

[2] One collection was qualitative only, and remaining 20 collections were qualitative and quantitative

slight and intermittent, only 4.7 percent of the population consisted of darters, and these fish were frequently absent in the collections. Stations 5.2 and 4.2 which were inhabited only intermittently by small numbers of the relatively tolerant species, yielded only one darter in all the collections. At Station 2.8, which is in the lower portion of the zone of recovery, three species of darters were collected. These darters comprised 1.9 percent of the total collections. They were present intermittently, and were taken in only 10 of 20 collections. At Station 1.0, six different species of darters were collected, and although they were not dominant, comprising only 1.2 percent of the collections, they were found in 12 of 20 collections. No black bass were ever taken at Stations 5.2 and 4.2, and only 4 bass were collected at Station 2.8, whereas at Station 1.0, where the stream had fully recovered from the pollution, 72 black bass were collected. Spawning of black bass seems to have been successful in this area in the spring of 1951.

The presence of black bass and darters is good evidence that organic pollution is not a major limiting factor in an area. However, the use of these species as indicators of favorable stream conditions is limited to specific areas because they do not occur in some types of waters and generally have a scattered geographic distribution. It is perhaps safer, therefore, to rely on the numbers of species of all fish as indicators of the degree of pollution rather than on the presence or absence of a particular species. Even so, careful judgment must be exercised because the number of species normally present in unpolluted streams in different localities with different physical and chemical characteristics varies widely.

DISSOLVED OXYGEN AND FISH DISTRIBUTION

It is an all too common belief that the only deleterious effect of domestic sewage and similar organic wastes on streams lies in the depletion of the dissolved oxygen in the water to a level that results ultimately in the death or dispersal of the normal biota. If the wastes are sufficiently diluted, so that the biochemical oxygen demand can be satisfied while sufficient dissolved oxygen remains to satisfy the needs of the various organisms present, the pollution with sewage is deemed harmless to aquatic life. Our observations on Lytle Creek indicate that the problem is somewhat more complicated than is commonly believed.

In Lytle Creek during the winter and spring months, stream flow exceeds 6 cubic feet per second, water temperatures are low, and the oxygen concentrations at all stations approach saturation. On the basis of chemical and physical criteria ordinarily employed, the water would be judged to be free from pollution harmful to fish, but the fish did not move during the winter into the areas which were unacceptable to them during the summer. On the contrary, it seemed that the fish found conditions poorer at some of the stations during the winter. Collections made in February and March 1952 failed to yield any fish at Stations 5.2 or 4.2, and only 8 species were taken at Station 2.8. It must be noted, however, that a few darters were present at Station 2.8 in March.

Factors other than the lack of oxygen have made these stations relatively unattractive to fish in winter. Possibly the sewage fungi, bacteria, protozoans, and other pollution-tolerant organisms, which became abundant on the stream bottom as far downstream as Station 2.8 during the colder winter months crowd out those bottom invertebrates that serve as food for some fish. In spite of the presence of the blanket of sewage-tolerant organisms, some fish species remained abundant at Station 2.8; namely, the creek chub and the common sucker, which suggests that food was not a limiting factor for these forms.

During the late spring and throughout the summer and early fall of 1951, the volume of flow fell to about 1 cubic foot per second, while the time required for the sewage effluent to flow from the treatment plant to the mouth of Lytle Creek increased greatly. Water temperatures rose as high as 87° F. and oxygen concentrations became low in the 4-mile section of the stream immediately below the sewage-treatment plant. Definite pollutional zones became established in the late spring and lasted throughout the exceptionally dry summer. Although the oxygen values at all stations sometimes dropped at night to levels which are generally considered to be low, the number of species did not always seem to be influenced by the minimum dissolved-oxygen concentrations. Although the minimal oxygen content at Station 2.8 was sometimes similar to that at Station 4.2, the collections at Station 2.8 always indicated an abundant mixed-fish population whereas the fish were never abundant or varied at Station 4.2. In May and August 1951, for example, the dissolved oxygen at Station 2.8 was as low as 1.6 p.p.m., but large and varied fish populations were collected and observed there. During May 1951, the water at Station 4.2 had a minimum nocturnal dissolved-oxygen content of 1.0 p.p.m.; yet the best collection of the entire series at that station was made at that time. The maximum dissolved-oxygen value observed in daytime during this period was 18.1 p.p.m. These observations may indicate that fish can survive a rather severe decline of the oxygen content at night, if adequate levels are maintained during the day.

Unquestionably a certain minimum of dissolved oxygen is necessary for the survival of fish, but our findings on Lytle Creek prove that mixed-fish fauna can occur where dissolved-oxygen values are often well below 5 p.p.m. for periods of several hours.

Although the studies on Lytle Creek demonstrate a fairly good correlation between the size and composition of the fish populations and degree of purification of the stream, it is apparent that dissolved oxygen is only one of several factors influencing fish survival in polluted streams. Although the lack of dissolved oxygen in the areas immediately downstream caused fish to be absent during the summer periods of low flow, oxygen lack was certainly no factor in their distribution during the winter months or during periods of high stream flow. Furthermore, a mixed-fish fauna consisting of over 30 species of fish was found at Station 2.8 where the oxygen fell well below the commonly accepted standard of 5 p.p.m. for several hours during the night. However, game fish were found at this station during only a portion of the time and were never as abundant as they were at Station 1.0.

ACKNOWLEDGMENTS

The advice, assistance, and constructive criticisms of Public Health Service personnel and others have been of great benefit in this investigation. Rendell Rhoades and Milton B. Trautman of the Ohio Department of Natural Resources and the Ohio State University most obligingly compiled a list of the fish species they had previously collected in Clinton County, Ohio. This list was of great value in confirming our identifications. Dr. Shelby D. Gerking of Indiana University made some valuable and pertinent suggestions. Personnel of the Environmental Health Center who helped with the collections were: W. C. Howard, Donald R. Robertson, Ramon Schulte, Fred Crates, George Paine, Harold Walters, and Earl P. Floyd. The suggestions of Drs. Peter Doudoroff and Clarence M. Tarzwell were of great aid in planning the project and preparing this manuscript.

LITERATURE CITED

Gaufin, Arden R., and C.M. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Pub. Health Rep., Vol. 67, No. 1, pp. 57-64.

Gerking, Shelby D. 1947. The flowing stream -- one more world. Purdue Univ. Ext. Ser. No. 64. Proc.

Third Ind. Waste Conf., Eng. Bull., Vol. 31, No. 6, pp. 27-33.

Jackson, H.W., and C. Henderson. 1942. A study of the stream pollution problem in the Roanoke, Virginia, metropolitan district. Part II - Macroscopic invertebrate and vertebrate faunae. The toxicity of viscose effluent to fishes. Bull. Va. Polytech. Inst., Vol. 35, No. 10, pp. 91-120.

Thompson, D.H., and F.D. Hunt. 1930. The fishes of Champaign County -- a study of the distribution and abundance of fishes in small streams. Ill. Nat. His. Surv., Bull. 19, Art. 1, pp. 1-101.

Reproduced With Permission From:
THE PROGRESSIVE FISH-CULTURIST
20(1958) : 45-46

THE EFFECTS OF ACID MINE POLLUTION ON THE FISH POPULATION OF GOOSE CREEK, CLAY COUNTY, KENTUCKY

William R. Turner

Department of Fish and Wildlife Resources
Frankfort, Kentucky

Goose Creek, a tributary of the South Fork of the Kentucky River, was polluted on April 2, 1957, by acid waters from an operating coal mine. The mine responsible for the pollution is located about 4 miles from the mouth of Island Creek, which empties into Goose Creek 2 miles below the town of Manchester.

Sixteen and three-tenths miles of the stream was reportedly polluted, effecting a fish kill over the same area.

On April 22 and 23 an electric shocker was employed on Goose Creek in order to determine the extent of the reduction of the fish population. During the 3-week period between the time that the pollution occurred and the time that the study was begun, the water level had risen and subsided once.

The standing fish population was measured by shocking two similar areas, one above and one below the source of pollution, and comparing them.

A total area of 2.07 acres above the mouth of Island Creek was sampled. This area produced 126.9 pounds of fish, or 61.3 pounds per acre (Table 1).

Below the mouth of Island Creek, a total area of 3.44 acres was sampled. The total weight of fish re-

TABLE 1.--Fish population of Goose Creek in an unpolluted area above Island Creek on April 22, 1957

Species	Number		Length (inches)	Weight (pounds)	
Game Fish---------		17	-----------		15.0
Largemouth bass-	3		10.[illegible] - 13.8	3.1	
Kentucky bass---	1		8.6	.3	
Smallmouth bass-	13		5.[illegible] - 1[illegible]	11.6	
Pan Fish----------		72	-----------		21.8
Rock bass-------	48		4.2 - 10.8	20.3	
Longear sunfish-	24		3.4 - 4.9	1.5	
Rough Fish--------		224	-----------		78.5
Freshwater drum-	1		12.2	.8	
Golden redhorse-	193		5.0 - 13.7	6[illegible].2	
Hog sucker------	23		5.0 - 13.1	[illegible].4	
Yellow bullhead-	2		8.8 - 9.8	1.1	
Flathead catfish	5		7.0 - 15.3	4.0	
Mixed Minnows-----	200		1.5 - 3.0		11.6
Totals-------	513		-----------		126.9

TABLE 2.--Fish population of Goose Creek in a polluted area below Island Creek on April 23, 1957

Species	Number		Number (inches)	Weight (pounds)	
Game Fish----------		4	-----------		5.3
Largemouth bass--	1		13.9	1.6	
Smallmouth bass--	3		7.5 - 15.9	3.7	
Pan Fish-----------		18	-----------		2.2
Rock bass--------	3		6.9 - 8.1	1.3	
Longear sunfish--	15		3.0 - 5.6	.9	
Rough Fish---------		44	-----------		10.6
Golden redhorse--	25		5.0 - 9.0	2.1	
Hog sucker-------	10		4.0 - 7.1	.8	
Quillback--------	2		8.0 - 9.3	.7	
Longnose gar-----	4		18.7 - 21.1	2.5	
Flathead catfish-	3		12.9 - 17.8	4.5	
Mixed Minnows------		23	1.5 - 3.0		.4
Totals--------		89	-----------		18.5

covered in this area was 18.5 pounds, or 5.38 pounds per acre (Table 2).

It was estimated by the crew of 6 men conducting the study that 70 percent of the shocked fish were recovered.

The total poundage of fish in the area above Island Creek was nearly 12 times as great as the total poundage of fish in the polluted area.

In order to estimate the extent of damage to the game- and pan-fish population, an actual creel value was placed on each fish of catchable size. The sum of $1.22 per fish was calculated from creel-census cards received from 10,000 fisherman during 1955.

The damage to the rough-fish population was determined by placing a commercial value on the estimated poundage of fish destroyed. The sum of 20.6 cents per pound was calculated by averaging the commercial sales price of rough fish taken by trot lines. The average prices charged by the commercial fisherman using nets, seines, and snag lines were excluded because these methods are not employed in Goose Creek.

The estimated creel value of fish destroyed was $2,212 for game fish, and $9,268 for pan fish. The estimated commercial value of rough fish destroyed was $1,845 (Table 3).

The total economic loss that resulted from the pollution of Goose Creek was estimated to be $13,325. This is a minimum estimate of damage in that linear measurement only was used in computing the polluted segment of the stream, and no corrections were made to compensate for the increasing fish population that is normally encountered when approaching the mouth of a creek. Also, muskellunge were not obtained in the shallower areas sampled, but the fish kill showed that they had been present in the deeper downstream holes.

TABLE 3.--Estimated economic loss in 15.3 miles of Goose Creek, as determined by creel value and commercial value of species killed

	Calculated number of game fish	Calculated number of pan fish	Calculated poundage of rough fish
Polluted area--------------------------------------	298	1,343	780
Unpolluted area------------------------------------	2,111	8,940	9,747
Number of catchable fish destroyed-----------------	1,813	7,597	----------
Creel value of fish killed-------------------------	$2,212	$9,268	----------
Poundage of commercial fish destroyed--------------	----------	----------	8,957
Commercial value of fish killed--------------------	----------	----------	$1,845
Total damage---------------------------------------		$13,325	

Chapter V

RELATIONSHIP OF POLLUTION TO MAN

Reproduced With Permission From:
SEWAGE WORKS JOURNAL
21 (1949): 110-114

WATER QUALITY REQUIREMENTS FOR RECREATIONAL USES*

Albert H. Stevenson
Sanitary Engineer, U. S. Public Health Service

Increased public interest in water pollution during the past few years has resulted in active consideration of this problem by many state legislatures and in the passage of more adequate control laws by several of the states. The Commonwealth of Pennsylvania was one of the first states in recent years to adopt such measures. This interest in water pollution reached a climax with the enactment of a Federal Water Pollution Control Law and with the ratification of the Ohio River Valley Water Sanitation Compact by eight states in June, 1948.

Both of these developments should be of particular interest to the municipalities and industries in Pennsylvania. This symposium for presenting fundamentals of water pollution abatement for industry also becomes most appropriate at this time. In discussing these fundamentals it is apparent that water quality requirements for various water uses are basic. However, with the many types of water use and the large number of examinations employed to determine water quality, these requirements cannot be simplified to the extent desirable. Often the emphasis must shift from one type of water quality measurement to another with what appears to be only a moderate change in the water use. The water quality requirements for recreational uses perhaps emphasize this point the most.

TYPES OF RECREATIONAL USE

For this discussion recreational use has been divided into three classifications. First, swimming and bathing in natural bodies of water such as lakes, ponds, streams, rivers and tidal waters, or in impounded waters such as reservoirs or lakes. Second, fishing in natural or impounded bodies of water involving as it does the sustenance, growth and propagation of the higher forms of native aquatic life. Third, boating on or camping along bodies of water used solely for boating and similar purposes.

In determining the factors which should be considered for water quality requirements for each of the types of recreational activity, it is obvious that for swimming and bathing, public health aspects are paramount. However, aesthetic considerations are also of great importance in this connection. Similarly, the support of native fish life depends upon the maintenance of a favorable physical, chemical, and biological balance in the body of water together with an absence of elements which are detrimental to fish propagation. The major considerations for the boating and camping use of water are the prevention of nuisance conditions which result in objectionable odors, floating matter, or the deterioration of the protective coatings of the hulls of floating craft.

BATHING WATERS

The particular items of most importance in determining the suitability of bathing waters for general use are freedom from concentrations of pathogenic organisms which cause illness to human beings, absence of substances which are toxic to man, and absence of objectionable visible or foul-smelling substances. There are a number of standards of bathing water quality in existence in various parts of the country. Many state health departments have adopted standards for outdoor bathing places. Furthermore, standards or limiting values of various water quality measurements have been recommended or adopted by interstate groups for the Ohio River Basin, the Tennessee River Basin, the Great Lakes and Upper Mississippi River Areas, and the Potomac River Basin, to name a few. In recommending limiting densities for bathing and recreational use in the Ohio River Basin, the results of the Winslow-Moxon studies at New Haven, Conn., and Scott's classification based upon studies of Connecticut bathing areas along the shore of Long Island Sound were used since they represented the best data then available.

All the standards have some items in common. All are based chiefly upon the bacteriological quality as measured by the density of coliform organisms. These organisms are found in the intestinal tract of man and therefore serve as an indicator of the presence of potentially pathogenic organisms. Thus the coliform group is used as a bacterial indicator of sewage pollution. However, the group also contains organisms which are found naturally in the soil. Most of the standards are based upon the correlation of bacteriological data and such chemical and observational data as are mentioned, with the sanitary survey of sources of pollution which may affect the body of water under study. The absence of toxic substances, free acids, floating solids, scum, oils, tars, grease, and preventable sludge deposits also is frequently listed.

---

*Presented at Annual Conference of the Pennsylvania Sewage and Industrial Wastes Association, State College, Pa., August 26, 1948.

The major points of difference in the important bacteriological requirements of the standards are primarily due to the fact that there was little or no epidemiological data available at the time most of them were adopted. Therefore, the limiting coliform densities selected were frequently those attainable in the area where surveys were being made. The coliform densities vary from a most probable number of 50 per 100 ml. in the Tennessee Valley ("Studies of the Pollution of the Tennessee River System II," TVA, 1945, p. 271) to 2,400 per 100 ml. in New York City (Board of Health Declaration of Policy dated July 9, 1948), with most standards in the 500 to 1,000 per 100 ml. range.

However, the method of arriving at this density varies considerably and significantly. In some standards it is the maximum permitted, presumably in the determination on any one sample. In others, it is the average of a series of samples, but the type of average, arithmetic or logarithmic, is not stated. In some standards an occasional overrun of the limiting concentration is permitted and in one state 20 per cent of the results may exceed 1,000 per ml. This last appears to be a realistic approach to a water quality requirement.

In the 1948 Report of Joint Committee on Bathing Places, the members of the committee representing the Conference of State Sanitary Engineers and the American Public Health Association comment as follows with regard to limiting coliform concentrations at outdoor bathing places: "It is emphasized that final classification of bathing waters should depend largely upon sanitary survey information. Bacteriological analysis findings should be used as a guide. Some areas of the country are reported to be able to meet bacterial standards for natural surface waters with maximum limits of coliform indices (as used herein) of not more than 240 to 500 per 100 ml. Such excellent quality is, of course, greatly to be desired. However, experience indicates that other sections of the country, especially those located in more densely populated areas and among cultivated lands, cannot maintain such conditions.

"Waters showing a concentration of more probable numbers of coliform organisms of less than 1000 per 100 ml. are considered in most such areas to be fairly acceptable for bathing unless the sanitary survey discloses immediate dangers from human sewage pollution; however, it must be admitted that bathing beaches where the content of coliform organisms runs as high as 1000 per 100 ml. according to the Phelps index or 2400 per 100 ml. on the basis of most probable numbers, or sometimes even higher, have been used without reported evidence of illness, and this limit of 1000 per 100 ml. according to the Phelps index is still employed as a criterion of acceptability in some states. The trend is, of course, to reduce bacteria counts where possible by sewage treatment if human sewage is a threat, and the attainment of reasonable progress in this direction is to be hoped for. While bathing areas should always be free of visible forms of sewage matter that might contain pathogenic organisms, serious pollution may be present in many waters where treatment of sewage removes visible evidence of sewage solids but does not eliminate dangerous concentrations of bacteria."

The U. S. Public Health Service has not recommended a nationwide standard for bathing water quality although limiting coliform densities were recommended for the Ohio River Basin, as was previously noted. However, the Cincinnati Station of the Service is at present actively participating in a joint bathing water study at the southern end of Lake Michigan with the Wisconsin, Illinois and Indiana Boards of Health and with five metropolitan Chicago and Cook County agencies. Nine laboratories are cooperating in this project, the objectives of which are: (1) to determine the physical, chemical, and bacteriological quality of the waters tributary to Lake Michigan public beaches between Milwaukee, Wis., and Michigan City, Ind., (2) to study the enterococcus (streptococcus) group of organisms together with the coliform group as indicators of sewage pollution, (3) to assess the effect of the bathing load itself upon the water quality at heavily used beaches, and (4) to obtain epidemiological evidence which may lead to the development of a sound bathing water standard.

In connection with the epidemiological objective, two local beaches have been selected in Chicago for special study. Sanitary surveys of each beach are being made and samples of water are collected under various conditions of wind, weather, and temperature for bacteriological examination. Bathing load observations are made also. Adjacent population groups numbering over 1,000 families in each area and representing persons most likely to use these particular beaches are being studied. With the assistance of a small staff of field workers, each household is maintaining a daily swimming and illness record of individual members of the family for a 2-month period. It is hoped that from a review of these data at the completion of the study any significant rise in the illness incidence of swimmers above that in the non-swimmer or occasional-swimmer control group may be noted for correlation with the respective beach water qualities.

This epidemiological study in itself is a limited one and is confined to two points in one area of the country. It is believed that additional studies of this type are needed in other areas where different water quality conditions prevail and other closely built-up residential areas exist which would make such studies practicable.

It is not the purpose of this paper to suggest a standard of bathing water quality. However, it is believed that the elements which should be considered in setting up water requirements for bathing can be presented. The determinations which appear to be of most significance on the basis of present knowledge are:

(1) Bacteriological--the determination of the concentration of organisms of the coliform group, with the possibility that other groups, such as enterococcus, may furnish a valuable supplement to the coliform group.

(2) Biological--examinations indicating the absence of toxic algal blooms and cerceriae.

(3) Chemical--examinations indicating the absence of toxic substances, free acids, and concentrations of chemicals which produce objectionable tastes or odors; also the presence of dissolved oxygen (several existing standards indicate that the dissolved oxygen should exceed 5.0 p.p.m.).

(4) Physical--observations indicating the absence of oils, tars, greases, scum, floating solids, and debris, except that which comes from natural sources; preventable sludge deposits; and turbidity (limiting amounts of from 10 to 250 p.p.m., except after heavy rains, have been noted in several standards). The latter item is of importance from the standpoint of safety against drowning and as a check on the effect of wave action.

In considering the suitability of bathing waters for use, it is essential that the above results, representing water quality, be correlated with the sanitary survey of potential sources of pollution and with the bathing load.

WATERS USED FOR FISHING

Pertinent factors necessary for the sustenance of native fish life are the presence of adequate concentrations of dissolved oxygen and adequate sources of fish food in the waters, as well as the absence of organic sludge blankets which are detrimental to spawning and the absence of substances which are toxic to fish. The minimum concentration of dissolved oxygen which will support and propagate most types of native fish has been found by both the U. S. Bureau of Fisheries (Bulletin Number 22) and by the U. S. Public Health Service (Supplement F, Part II, Ohio River Pollution Control, p. 1275) in independent studies to be about 5 p.p.m., although some of the more resistant varities will thrive at slightly lower concentrations.

In conjunction with the harvesting of shellfish which may be eaten raw, another important consideration is the bacteriological quality of overlying waters from which shellfish are taken. Since the epidemic of typhoid fever in 1924 which was traced to the consumption of raw shellfish, there has been a much greater emphasis on shellfish sanitation. The present standard for the bacteriological quality of overlying waters in approved areas is a most probable number of 70 coliform organisms per 100 ml. based upon the median of all the results at a sampling point. However, bacteriological requirements for shellfish and shellfish growing areas are not static. The U. S. Public Health Service has recently established a special laboratory at the Woods Hole Oceanographic Institution in Massachusetts for further studies on this subject.

Various water quality standards suitable for the sustenance and propagation of native fish life have been proposed or promulgated by several agencies concerned with water pollution control in the Ohio River Basin, Tennessee Valley, Potomac River Basin, streams of West Virginia, and shellfish-growing areas in coastal waters. As might be expected, these standards are not completely uniform; however, the variations in values for the several determinations are not great. The range of existing water quality requirements for the continued maintenance of native fish life encountered in these several standards is:

Dissolved oxygen: 5.5 to 6.5 p.p.m. monthly average; 5.0 p.p.m. daily minimum.
5-day B.O.D.: 4.0 to 5.0 p.p.m. minimum observation; 1.5 to 4.0 p.p.m. monthly average; 3.0 to 6.0 p.p.m. daily maximum.
Coliform bacteria (M.P.N.)*: 70 per 100 ml. or less.
Sludge deposits: none to moderate localized.
pH: 6.5 to 8.6; 5.0 to 9.5.
Other conditions: no toxic substances, oils, tars or free acid at any time; no floating solids or debris, except from natural sources; occasional to no taste producing substances.

It will be noted that there is no measure of the quality of fish food available. It is assumed that if the quality is brought within the limits prescribed the chemical constituents and the flora and fauna native to the water will support the fish life which is normal to the area.

WATERS USED SOLELY FOR BATHING AND CAMPING

There appears to be few water quality requirements in effect which cover this particular water use. As has been mentioned, the major factors are the prevention of nuisance conditions and the protection of the coatings and hulls of floating craft from deterioration. An example of one set of standards which are applicable to this situation is the minimum quality criteria for Class D waters for streams in the Potomac River Basin, approved August 8, 1946. The criteria for Class D waters call for only the general sanitary requirement that there be no nuisance condition. The specific criteria are:

---

*For native shellfish-growing areas only.

pH: 6.0 to 8.5.
5-day B.O.D.: 3.0 p.p.m. monthly average; 5.0 p.p.m. maximum observation.
Dissolved oxygen: 4.0 p.p.m. monthly average; 3.0 p.p.m. minimum daily average; 2.0 p.p.m. absolute minimum.
Other conditions: no toxic substances, oils, tars, or free acid at any time; no floating solids or debris, except from natural sources; slight localized sludge deposits allowed, if unpreventable; no offensive odors.

It will be noted in these criteria that no free acid is permitted and that the pH range is limited to prevent the occurrance of highly acid or highly alkaline waters. The limiting oxygen requirements will assure aerobic conditions and reduce the occurrance of odors. Similarly the elimination of various floating substances should make the appearance of the water acceptable from an aesthetic standpoint.

CONCLUSIONS

In this paper no attempt has been made to discuss specific industrial wastes and their effects upon the recreational uses of water. Practically all wastes, either treated or untreated will affect one or more of the measurements used for determining quality. Furthermore, although the toxicity of many wastes has been studied, little is known of others. Then too, the discharge of particular substances to a watercourse brings up the difficult problem of limiting such discharges from individual plants sufficiently to maintain a sub-toxic level in all parts of the watercourse at all times.

In addition to further toxicity studies, it is suggested that more epidemiological studies in the vicinity of bathing areas, bacteriological research on pollution indicators, and biological investigations into the effects of microscopic plants and animals on bathers should be carried out with due regard to geographical considerations.

Reproduced With Permission From:
PROCEEDINGS FIRST OHIO WATER CLINIC
OHIO STATE UNIVERSITY ENGINEERING SERIES
BULLETIN 147 (1952): 34-39

WATER POLLUTION, ITS EFFECT ON THE PUBLIC HEALTH

John D. Porterfield, M.D., M.P.H.

Director, Ohio Department of Health, and
Chairman, Ohio Water Pollution Control Board

When we consider the waters of Ohio from the viewpoint of the public health, we must concern ourselves immediately with the problem of pollution. In general, we have only occasional and isolated problems of quantity of water from the health viewpoint; we do have a serious problem of quality.

A tremendous amount of raw sewage from municipalities and untreated wastes from industries have been and are still being poured into our streams and lakes everyday. We have approximately 100 communities, representing a population of more than one million, which are discharging over 100 m.g.d. of raw sewage into streams. These communities have no sewage treatment plants at all. We have another 250 communities, with a population of 3 1/2 million, which have some form of sewage treatment plants, but many of these plants are not adequate. These communities discharge another 350 m.g.d. of wastes into our watercourses much of it not properly treated. We know also of some 500 industries of significant size which are discharging wastes directly into our surface waters. The total amount of wastes from these industries is comparable to the total amount of wastes from municipalities, practically doubling the pollution load in our waterways.

The total amount of wastes being discharged into our streams and lakes therefore approaches a billion gallons daily, only a small portion of which is being adequately treated at the present time. The time is at hand when we should have a strong and coordinated program of pollution abatement!

INDUSTRIAL WASTES

We can classify the principal types of industrial wastes into about 20 groups. But even within one group, there is tremendous variation in the volume and strength of the wastes.

In the paper industry, for example, different processes used in producing different types of paper result in wastes varying from 4000 up to 85,000 gallons for each ton of finished paper.

In the canning industry, the amount of wastes will vary from 750 to 25,000 gallons for each 100 cases of product.

Here are a few other examples indicating the waste potential in different industries:in oil refining, 700 to 1000 gallons of wastes per barrel of oil; in coke production, 3600 gallons per ton of coal carbonized; in woolen mills, up to 240,000 gallons for each 1000 pounds of finished goods; in meat packing, 550 gallons for each hog butchered; in milk processing up to 340 gallons of wastes for each 1000 pounds of milk and cream; in poultry dressing, 2200 gallons for each 1000 pounds live weight of fowl; in beet sugar production, 3000 gallons of wastes per ton of beets.

Untreated wastes of industries and raw sewage from municipalities have placed such a pollution burden on our streams that many have long since lost the normal ability to purify themselves.

PERCENTAGE

The actual percentage of pollution in any stream at a specific location will vary from season to season, according to stream flow. For health purposes, obviously, the amount of pollution must be measured at periods of lowest flow.

Directly downstream from many municipalities, the pollution load during such periods frequently amounts to half or more of the total flow, depending of course on the size of the municipality and the size of the stream.

But even a river as big as the Ohio becomes polluted in almost unbelievable proportions. At Cincinnati, for example, for every gallon of water flowing past the city in some seasons, there is an equivalent of one quart which might have been drawn directly from a sewer. From this nauseating mixture, Cincinnati must concoct her water for drinking, cooking, and bathing.

HEALTH

The effect of this gross pollution of our waterways on health is both direct and indirect.

INDIRECT EFFECT

One indirect effect, sometimes forgotten, stems from economic factors. When pollution becomes so bad that industrial development is curtailed, both incomes and health standards of individual workers are lowered. Good jobs mean better income; better income means better health for workers and their families. Continued pollution of our streams can actually reduce the number of jobs. Some companies have chosen not to establish plants in Ohio because investigation showed our water supply to be poor in industrially favorable locations.

Another indirect effect on health, and one which is better known to most of us, comes from the loss of recreational areas. Outdoor recreation is healthful. The opportunity for such recreation has been reduced tremendously by the despoilment of streams and lakes. It has been necessary to ban swimming in most rivers and streams and even in many locations on Lake Erie. Fish have been destroyed in many streams, causing damage to both a hobby and an industry.

DIRECT EFFECT

But pollution of our state's waters directly affects health in many ways. In the first place, we must make note of the fact that 75 per cent of the people in Ohio obtain their drinking water from surface waters. Pollution of these waters with a billion gallons daily of sewage and industrial wastes presents a problem of vital concern to health authorities.

TREATMENT

The first danger we face is the transmission, through polluted waters, of such diseases as typhoid fever and the dysenteries. Our major attack on this problem, to date, has been in the effort to treat the water for domestic use rather than to reduce the pollution at the source. This attack has been so successful that most persons take safe drinking water for granted in the cities and villages which have water treatment works. Actually, we may be placing too much confidence in these treatment plants.

CHLORINATION

As pollution of streams has increased through the years, the only major change in water treatment has been intensified chlorination. But chlorination is a thin wall of defense, subject both to human error and breakdown of equipment. Bacteriologic control is dependent on a post check, which means that our tests reveal the condition of water which went into our homes 48 hours previous to the tests. Chlorination also has its limitations, among them its inability to destroy certain intestinal irritants, either derived from sewage or industrial wastes or produced by chemical action from them.

Dr. M. V. Veldee, making a study for the Public Health Service along the Ohio River during periods of excessively low flow in 1931, reported that extensive outbreaks of gastroenteritis occurred successively in cities and villages from Wheeling to Louisville. He said the evidence suggested that these intestinal disturbances resulted from the presence of some soluble substance or combination of substances formed when the Ohio River was in extremely long periods of pool stage. These substances were not removed by the ordinary processes of water treatment, nor were they revealed by the usual tests for a potable and safe water.

One question naturally arises. How much more can we pollute our streams with sewage and industrial wastes before such conditions begin to appear at seasons other than low river flow?

TOXICITY

Another question concerns the cumulative toxic effects on the human body of chemicals and metallic particles reaching the streams in industrial wastes and not being removed by water treatment works. Not much is known about these possible dangers, but they are under study now at Kettering Laboratory, and should be at many other research centers. We need to know the effect of modern wastes on human physiology if we are to protect the public health. We need knowledge also to protect industry when innocuous wastes are incorrectly accused of causing ill health. Among answers being sought are the effect of sodium ions on cardiac patients, the effect of chlorides on persons with hypertension, and the possible presence of cancerogenic agents.

Toxicity control in water-treatment works is dependent today primarily on checks of potability: taste, odor, and appearance. Chemical tests must be improved. Also we must seek the removal of potentially dangerous elements from industrial wastes before these wastes are discharged into our streams.

TASTE

Some wastes, such as phenols, cause an unpleasant taste in drinking water. Frequently chlorination merely intensifies the unsatisfactory flavor. Obviously, optimal health may be affected if many persons choose to reduce their intake of water because they dislike the flavor of it. Many persons drink too little water. The average person needs from three to five quarts of water per day, according to estimates depending on the amount and type of activity in which he engages. To consume this large amount of water requires considerable effort. The task is not made easier when the taste or odor is objectionable.

Obviously also, the protection of water by municipal purification processes does not remove the danger of germs being carried directly by insects from our impure stream to our food supplies. In addition, cattle, dogs, and other animals may carry bacteria and filth from polluted streams into milk supplies and into homes, not to mention possible effects of the germ-carrying on the animals themselves.

ODOR

Families whose homes are located along these polluted streams are further handicapped by seasonal discomfort from noxious odors. Such discomfort, and the feeling of frustration in dealing with it, can cause and has caused neuroses to develop. In fact, one court judgement was handed down on the basis that harmful physiological effects from such noxious odors had been demonstrated.

Briefly summarizing, we note the following effects on health from our severe water pollution: (1) transmission of enteric diseases by water inadequately treated, (2) disease transmitted by insects from polluted streams, (3) individual water intake harmfully reduced by water potability, (4) possible toxicity of chemical and metallic wastes, (5) neuroses caused by noxious odors from polluted streams, (6) disease spread by cattle and other animals having access to polluted streams, (7) loss of extensive recreational areas due to pollution, and (8) economic changes due to pollution.

ACTION

This list is a severe condemnation of present conditions. But we may take encouragement from the fact that Ohio now is prepared to take more effective steps than ever before toward the control and reduction of water pollution.

OHIO WATER POLLUTION CONTROL ACT

The new Ohio Water Pollution Control Act passed by the 99th General Assembly in 1951 offers real hope for improvement. This Act has created a separate State Water Pollution Control Board, combining in one agency the interests of many groups concerned with the major problem and including the Department of Health, the Department of Natural Resources, the Department of Commerce, and many industries and municipalities.

This Act makes it unlawful to pollute any of the waters of the state, and describes pollution as "the placing of any noxious or deleterious substances in any waters of the state which renders such waters harmful or inimical to the public health, or to animal or aquatic life, or to the use of such waters for domestic water supply, or industrial or agricultural purposes, or for recreation."

Under this law, municipalities and industries will be required to obtain permits for the discharge of sewage, industrial wastes, or other wastes into the waters of the state. The volume and strength of such discharges, determined by the degree of treatment, will be specified in conditions attached to each permit.

The policy of the Water Pollution Control Board will be to issue these permits for specific periods and to re-issue permits only after applicants have demonstrated real progress toward the abatement of pollution. The Board is also empowered to (1) conduct programs of investigation, research, and education, (2) issue orders and regulations, (3) require the construction or modification of sewage and waste disposal systems, and (4) institute court action to compel compliance.

PROSPECTS

Correction of the pollution problem will not be achieved overnight, even with this new law. And in this industrial era, one cannot expect that all streams are restorable to the pristine beauty and purity that Daniel Boone found.

Many streams, however, can be made recreationally valuable again for fishing. Some can be made clean enough for swimming. All can be freed from noxious odors. Bacteria and undesirable chemical elements can be reduced to a point where temporary breakdown of a water-treatment works would not seriously threaten the residents of an entire community.

These improvements will be accomplished gradually--by education, largely by co-oporation, and by the application of legal procedure where necessary.

Reproduced With Permission From:
PLANT DISEASE REPORTER
40(1956): 681-687

POTENTIAL PLANT PATHOGENIC FUNGI IN SEWAGE AND POLLUTED WATER

Wm. Bridge Cooke[1]

The literature of sewage and waste disposal contains many references to the use of sewage as a source of supplemental irrigation water. References are increasing as studies are made in drier parts of the country where sewage and other wastes are of increasing interest in reclamation of water.

Skulte (1956) states: "Skillfully designed and properly managed sewage farming has been operated for decades in Europe, and for several years in some localities in the southwestern areas of the United States, without danger to personnel and without creating nuisance conditions. As a new science, irrigation with sewage effluent is still in the experimental stage and many problems need to be solved in the fields of bacteriology, biology, hygiene, soil structure and the physiology of plants."

State and federal agencies have been interested for many years in the use of sewage as an irrigation supplement. Most communities in the United States could use supplemental irrigation (Hunt, 1954) during annual periods of drought. Methods of reclaiming sewage for reuse in irrigation have been described by Hutchins, 1939, and are being studied by the State of California (Bush and Mulford, 1954; Merz, 1955). "Water", the Yearbook of Agriculture for 1955, shows in several articles both by Department of Agriculture personnel and by Public Health Service personal and under-current of interest in this problem. On the research level, certain recent studies indicate some of the problems involved. The effect of spreading rather than furrowing in irrigation technique has been investigated by Bush and Mulford (1954) and by Greenberg and Thomas (1954). A study by Henry et al. (1954) was designed to indicate the influence of sewage waters on mineral content, organic content, soil structure, and other aspects of soil quality. In such studies it has been found that the most important mineral problem is that of boron which sewage seems to add to soils.

In a popular article by Stone (1955) it was pointed out that sewage could be used for irrigation in a number of places. In addition to irrigating crop plants, he thought, it could be used for the irrigation of lawns and ornamental planting. Illustrations of both types of use are given in his article.

In his new book, "The Biology of Root-Infecting Fungi" Garret sums up his thesis with a statement to the effect that until a crop is grown in a soil free from disease organisms one cannot appreciate the resulting gains in crop values or the typical healthy nature of the plants that develop in such conditions. We may assume that this statement can also supply a contrast to soils into which disease organisms, however unapparent they may be, are dumped on a periodic and perhaps daily schedule.

Menzies (1954) has pointed out that certain plant pathogens have become more and more a problem to the plant pathologist as the practice of irrigation increases in the Columbia Basin. Those pathogens mentioned are airborne leaf and fruit diseases rather than waterborne soil fungi and either have been introduced with the crop or have migrated in much the same manner as other rusts, mildews, and host-restricted pathogens. His forecast that other diseases could become important in the region is of interest.

---

[1] In charge, Fungus Studies, Microbiology, Water Supply and Water Pollution Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati 26, Ohio.

Two of the fungi isolated occasionally from sewage and polluted water are *Fusarium oxysporum* and *F. solani*. Strains of both have been recovered at one time or another from domestic sewage used as "seed" in experiments at the Robert A. Taft Sanitary Engineering Center. To determine the degrading ability of sewage organisms for various types of industrial wastes, small amounts of such sewage are seeded into dilution water containing known quantities of the wastes or materials to be studied. In one such experiment, hydrocarbons in the form of motor oil were subjected to domestic sewage. During the period of observation two groups of organisms developed in large quantities, a protozoan and a strain of *F. oxysporum*. In a later experiment this strain of *F. oxysporum* was added to a suspension of motor oil in dilution water. After three weeks (Ludzack and Kinkead, 1955) the fungus had produced almost as much breakdown of the oil as had the domestic sewage which was tested at the same time in similar solutions. Similar sewage seed had been used to develop a slime on an experimental trickling filter in which hydrocarbons of airplane cleaning materials were treated. Upon plating the slimes it was found that a strain of *F. solani* was the predominant fungus present.

Prior to 1952, the presence of fungi in polluted water and sewage was considered by workers in the field of sanitary science to be an established fact but not worthy of special investigation. It was obvious that under certain conditions of pollution a stream or a trickling filter would develop growths thought to be fungi, some of which were identified as to species in occasional studies (Cooke, 1954a). No reference to the isolation or identification of plant pathogens as such from raw sewage or partially treated waters has been reviewed.

The routine study of a small polluted stream (Cooke, 1954c), of a large secondary-type sewage treatment plant at Dayton, Ohio (Cooke, 1954b) at monthly intervals, and of other habitats at less frequent intervals, yielded quantitative and qualitative data on the presence of soil fungi in these habitats. Various media were tested and techniques used which are common to soil microbiology (Cooke, 1954b). From such studies it appears that certain strains of species of soil fungi have become acclimated to living in aqueous habitats. Spore production is atypical when present, and certain tested species are able to use dissolved oxygen in competition with other sewage organisms for reduction of organic materials (unpublished data). Most of the species tested are able to produce their own vitamin supply, and all are able to utilize simple sugars and organic as well as ammonia nitrogen.

Garrett considers seedling blight and vascular wilt fungi as "ecologically obligate parasites"; that is, while they are able to grow along with other fungi on culture plates, in the soil they appear unable to compete with strictly saprobic fungi for the dead organic matter that occurs there. On the other hand, there is evidence that in sewage and related habitats they can reproduce, as indicated by the observations on degradation of hydrocarbons.

Table 1. Fungi of interest to the plant pathologist isolated from polluted water and sewage.

Alternaria tenuis
Aspergillus flavus
Aspergillus fumigatus
Aspergillus niger
Aspergillus terreus
Botrytis cinerea
Cephalosporium spp.
Chaetomium funicolum
Chaetomium globosum
Cladosporium cladosporioides
Coniothyrium fuckelii
    (and other sphaeropsidaceous fungi)
Curvularia lunata
Fusarium aquaeductuum (episphaeria)
Fusarium moniliforme
Fusarium oxysporum
Fusarium roseum
Fusarium solani
Gliocladium catenulatum
Gliocladium roseum
Mucor - several species
Penicillium brevi-compactum
Penicillium chrysogenum
Penicillium cyclopium
Penicillium digitatum
Penicillium expansum
Penicillium italicum
Penicillium martensii
Penicillium oxalicum
Penicillium rugulosum
Rhizopus nigricans and other species
Scopulariopsis brevicaulis
Stemphyllium consortiale
Trichoderma viride

Table 2. Occurrence of potentially plant pathogenic fungi isolated from Lytle Creek, Clinton Co., Ohio (Number of colonies in thousands per gram dry weight of four combined samples at each station in stream)

Station[a] and Fungus	1952									1953		
	Mar. 3	Apr. 17	May 15	June 12	July 10	Aug. 7	Sept. 18	Oct. 23	Nov. 26	Jan. 14	Feb. 25	Mar. 30
STATION 8.7												
Alternaria tenuis	8	0	0	0	0	0	0	0	0	0	0	0
Aspergillus fumigatus	172	20	124	30	10	24	8	40	20	30	20	20
Aspergillus niger	0	8	8	10	0	0	0	0	0	14	0	0
Fusarium oxysporum	0	0	0	18	0	0	0	0	0	0	0	0
Fusarium solani	0	0	10	0	0	0	0	0	0	54	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	34	0	0	0
Sphaeropsidales	8	38	108	0	0	0	100	140	0	342	0	0
STATION 7.6												
Alternaria tenuis	0	0	0	0	0	0	0	24	0	40	62	80
Aspergillus fumigatus	16	186	24	58	26	0	10	0	20	50	34	54
Aspergillus niger	8	0	16	10	0	0	0	72	520	444	50	0
Fusarium oxysporum	18	0	0	0	0	0	0	0	0	0	0	0
Fusarium solani	0	0	0	0	0	0	0	0	0	24	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	16	70	0	0	0
Sphaeropsidales	32	36	0	0	0	16	20	8	14	202	10	0
STATION 7.2 + 6.5												
Alternaria tenuis	0	0	8	0	10	0	0	100	34	44	0	28
Aspergillus fumigatus	218	44	178	30	0	32	10	3216	420	68	54	34
Aspergillus niger	0	0	0	8	0	0	100	0	0	10	0	0
Fusarium oxysporum	0	0	0	32	0	0	88	0	0	14	0	0
Fusarium solani	x	0	48	0	8	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	108	0	0	0	0	42	1530	20	0	0
Sphaeropsidales	16	336	26	0	0	100	8	24	30	474	114	0
STATION 5.2 + 4.2												
Alternaria tenuis	0	0	0	0	0	0	0	0	10	30	70	74
Aspergillus fumigatus	226	182	124	118	52	100	26	40	20	100	82	74
Aspergillus niger	0	26	0	0	10	0	100	0	10	0	0	14
Fusarium oxysporum	0	0	0	0	0	20	56	0	0	0	0	0
Fusarium solani	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	8	104	100	60	54	0	0
Sphaeropsidales	34	40	0	0	0	0	112	290	54	2868	164	10
STATION 2.8 + 1.0												
Alternaria tenuis	0	0	8	8	0	0	10	0	24	10	406	400
Aspergillus fumigatus	244	72	18	52	58	34	0	0	14	0	96	14
Aspergillus niger	500	8	0	0	200	8	108	0	14	0	48	0
Fusarium oxysporum	8	0	0	36	0	10	0	0	0	150	0	0
Fusarium solani	0	0	0	0	30	0	0	0	0	1974	0	0
Penicillium oxalicum	0	8	10	10	56	216	16	108	150	10	24	18
Sphaeropsidales	0	0	146	0	0	18	36	268	52	100	118	64

---

[a] Stations numbered according to distance in miles from mouth of creek. Stations fall in following pollution zones: clean water 1.0, 2.8, 8.7; zone of recovery 7.6, 4.2; Septic zone, 5.2, 6.5, 7.2; point of outfall from Wilmington, Ohio Sewage Treatment Plant, 7.2. See also Cooke(1954c) and Gaufin and Tarzwell (1952)

Table 2. Occurrence of potentially plant pathogenic fungi isolated from Lytle Creek, Clinton Co., Ohio (Number of colonies in thousands per gram dry weight of four combined samples at each station in stream)

Station[a] and Fungus	1952									1953		
	Mar. 3	Apr. 17	May 15	June 12	July 10	Aug. 7	Sept. 18	Oct. 23	Nov. 26	Jan. 14	Feb. 25	Mar. 30
STATION 8.7												
Alternaria tenuis	8	0	0	0	0	0	0	0	0	0	0	0
Aspergillus fumigatus	172	20	124	30	10	24	8	40	20	30	20	20
Aspergillus niger	0	8	8	10	0	0	0	0	0	14	0	0
Fusarium oxysporum	0	0	0	18	0	0	0	0	0	0	0	0
Fusarium solani	0	0	10	0	0	0	0	0	0	54	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	34	0	0	0
Sphaeropsidales	8	38	108	0	0	0	100	140	0	342	0	0
STATION 7.6												
Alternaria tenuis	0	0	0	0	0	0	0	24	0	40	62	80
Aspergillus fumigatus	16	186	24	58	26	0	10	0	20	50	34	54
Aspergillus niger	8	0	16	10	0	0	0	72	520	444	50	0
Fusarium oxysporum	18	0	0	0	0	0	0	0	0	0	0	0
Fusarium solani	0	0	0	0	0	0	0	0	0	24	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	16	70	0	0	0
Sphaeropsidales	32	36	0	0	0	16	20	8	14	202	10	0
STATION 7.2 + 6.5												
Alternaria tenuis	0	0	8	0	10	0	0	100	34	44	0	28
Aspergillus fumigatus	218	44	178	30	0	32	10	3216	420	68	64	34
Aspergillus niger	0	0	0	8	0	0	100	0	0	10	0	0
Fusarium oxysporum	0	0	0	32	0	0	88	0	0	14	0	0
Fusarium solani	x	0	48	0	8	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	108	0	0	0	0	42	1530	20	0	0
Sphaeropsidales	16	336	26	0	0	100	8	24	30	474	114	0
STATION 5.2 + 4.2												
Alternaria tenuis	0	0	0	0	0	0	0	0	10	30	70	74
Aspergillus fumigatus	226	182	124	118	52	100	26	40	20	100	82	74
Aspergillus niger	0	26	0	0	10	0	100	0	10	0	0	14
Fusarium oxysporum	0	0	0	0	0	20	56	0	0	0	0	0
Fusarium solani	0	0	0	0	0	0	0	0	0	0	0	0
Penicillium oxalicum	0	0	0	0	0	8	104	100	60	54	0	0
Sphaeropsidales	34	40	0	0	0	0	112	290	54	2868	164	10
STATION 2.8 + 1.0												
Alternaria tenuis	0	0	8	8	0	0	10	0	24	10	406	400
Aspergillus fumigatus	244	72	18	52	58	34	0	0	14	0	96	14
Aspergillus niger	500	8	0	0	200	8	108	0	14	0	48	0
Fusarium oxysporum	8	0	0	36	0	10	0	0	0	150	0	0
Fusarium solani	0	0	0	0	30	0	0	0	0	1974	0	0
Penicillium oxalicum	0	8	10	10	56	216	16	108	150	10	24	68
Sphaeropsidales	0	0	146	0	0	18	36	268	52	100	118	64

---

[a] Stations numbered according to distance in miles from mouth of creek. Stations fall in following pollution zones: clean water 1.0, 2.8, 8.7; zone of recovery 7.6, 4.2; Septic zone, 5.2, 6.5. 7.2; point of outfall from Wilmington, Ohio Sewage Treatment Plant, 7.2. See also Cooke(1954c) and Gaufin and Tarzwell (1952)

Table 3. Occurrence of potentially plant pathogenic fungi isolated from the sewage treatment plant, Dayton, Ohio. (Number of colonies in thousands per gram dry weight of sample.)

Station and Fungus	1952							1953				
	Apr. 3	June 16	July 17	Aug. 14	Sept. 25	Oct. 30	Dec. 10	Jan 29	Feb. 12	Mar. 19	Apr. 14	Aug. 19
IMHOFF TANK SCUM												
Alternaria tenuis			0	0	0	0	0	0	0	100	0	100
Aspergillus flavus			150	0	225	172	0	100	100	100	200	100
Aspergillus fumigatus			450	0	0	0	0	0	0	0	0	0
Aspergillus niger			600	0	450	258	200	200	100	200	100	0
Fusarium solani			150	0	0	258	0	0	0	0	0	0
Penicillium oxalicum			0	0	0	86	0	0	0	0	0	0
Sphaeropsidales			0	0	375	0	0	0	200	100	0	0
IMHOFF TANK SLUDGE												
Alternaria tenuis		0	0	0	0	0	0	0	0	170	0	170
Aspergillus flavus		0	260	0	0	0	0	0	340	0	0	0
Aspergillus fumigatus		0	390	0	260	300	170	0	0	0	0	0
Aspergillus niger		0	520	200	200	150	170	0	0	0	0	0
Fusarium solani		650	0	1170	0	1800	0	0	0	0	0	0
Penicillium oxalicum		0	0	520	650	0	0	170	0	0	0	0
SECONDARY DIGESTER SLUDGE												
Aspergillus flavus		0	220	0	0	0	0	0	0	0	0	0
Aspergillus fumigatus		110	220	0	110	0	0	0	150	0	0	0
Aspergillus niger		110	350	110	0	130	0	0	0	0	0	0
Fusarium solani		0	330	0	0	0	0	0	0	0	0	0
Sphaeropsidales		0	0	1100	4070	0	0	0	0	0	0	0
SCRAPINGS, HIGH-RATE FILTER SURFACE												
Alternaria tenuis	0	0	0	0	0	0	0	0	830	830	0	0
Aspergillus flavus	0	0	0	0	0	1540	0	0	0	830	0	0
Aspergillus fumigatus	630	0	2520	0	2520	0	0	0	0	0	0	1660
Aspergillus niger	0	0	630	1260	1890	770	1660	830	0	0	0	830
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	0	0	830
Sphaeropsidales	630	0	0	1260	0	0	0	0	0	1660	830	0
SCRAPINGS, STANDARD RATE FILTER SURFACE												
Alternaria tenuis	1260	0	0	0	0	0	1660	0	1660	830	0	2490
Aspergillus flavus	0	0	0	630	0	0	0	0	1660	0	0	0
Aspergillus fumigatus	1260	0	1890	0	0	0	0	0	0	0	0	0
Aspergillus niger	0	0	0	630	630	770	830	830	0	0	0	830
Sphaeropsidales	2500	0	0	4440	3150	1540	830	830	830	0	1660	1660
FRESHLY POURED SLUDGES												
Alternaria tenuis	0	0	0	0	0	0	0	0	144	0		72
Aspergillus flavus	0	0	54	0	0	124	0	0	0	0		0
Aspergillus fumigatus	0	0	108	162	0	62	216	0	0	72		0
Aspergillus niger	108	0	54	108	54	124	0	0	72	72		0
Fusarium solani	0	0	0	0	0	2	0	0	0	0		0
Penicillium oxalicum	54	0	0	0	0	0	0	0	0	0		0
Sphaeropsidales	0	0	0	1134	1180	62	0	0	72	72		0
MEDIUM-DRY SLUDGE												
Alternaria tenuis	0	0	0	0	30	0	0	0	0	0	0	0
Aspergillus flavus	0	0	0	0	0	0	0	0	120	0	0	0
Aspergillus fumigatus	0	0	0	0	0	34	40	80	40	0	0	0
Aspergillus niger	30	0	30	0	0	0	40	40	40	0	0	0
Penicillium oxalicum	0	0	0	0	0	0	0	0	0	40	0	0
Sphaeropsidales	0	0	0	0	0	34	0	0	0	0	0	0
AIR-DRIED SLUDGE												
Alternaria tenius	0	0	0	0	0	0	0	110	0	0	0	0
Aspergillus flavus	0	0	81	2511	81	0	0	0	110	0	0	0
Aspergillus fumigatus	972	0	0	0	38800	0	0	0	0	0	0	0
Aspergillus niger	0	81	0	405	0	0	0	0	110	0	0	0
Fusarium solani	0	0	0	162	0	0	0	0	0	0	0	0
Sphaeropsidales	0	0	243	0	12960	0	0	0	0	0	0	0
HEAT-DRIED SLUDGE												
Aspergillus flavus								368	0	368		0
Aspergillus fumigatus								0	184	920		184

In addition to the two strains of *Fusarium* species mentioned, other fungi have been found that are capable of attacking plants in the field, or plant products in the field, in harvesting, in storage, in transit, and on the market. Species in the accompanying list (Table 1) have been isolated from polluted waters and sewages. Some of the species on this list may be of little importance, but all have been cited in one reference or another as causes of disease in plants, associated with seed in storage, or causes of various types of rots and decays in storage, transit, and market. Most of the species in this list are mentioned prominently in "Plant Diseases", the 1953 Yearbook of Agriculture.

The fungi chosen for presentation in Tables 2 and 3 are among the commoner species that have been isolated from sewage and polluted water and have been listed in the literature of plant pathology as causing damage to growing plants or their products in the field, storage, transit, or market. *Alternaria tenuis* is listed in the broadest sense as including any isolate that has the morphological characteristics assignable to the species. Thus, several species of perfect fungi may be included in this category. The three species of *Aspergillus* cited are commonly associated with various types of storage, transit, and market decays, and some are commonly isolated from seeds. Several strains of *Fusarium oxysporum* and *F. solani* may be present in the region, but which of these are involved in the sewage isolations is not known. In order to determine this properly the cultivated and wild flora of the area should be screened for susceptibility to attack by these fungi. *Penicillium oxalicum* is reported to cause a seedling disease of corn, and it is also isolated on occasion from ears and seeds. Its occurrence in the area is of interest, since corn is one of the important crop plants there. The category "Sphaeropsidales" includes between 5 and 20 species, depending on the amount of variation permitted to occur within one species of imperfect fungi. Such genera as *Coniothyrium*, *Phoma*, *Spaeronema*, and *Ascochyta* are present, and one of the commoner species has been identified as *Coniothyrium fuckelii*.

The habitats mentioned in Tables 2 and 3 are described more fully elsewhere (Cooke, 1954b and c but may be briefly summarized here. Lytle Creek is a small stream in the Little Miami River basin and empties into Todd's Fork. It originates east of the City of Wilmington and flows through rolling farm land for about 10 miles. Before it received the effluent from the Wilmington sewage treatment plant, it was an intermittent stream. When the study was made on which Table 2 is based, Wilmington had a primary-type treatment plant in which solids were settled out and the liquids, sometimes treated chemically, were discharged into Lytle Creek. An activated sludge plant has since been installed, and zonation on the creek is probably much different because the stream is receiving much less polluted water. The sewage effluent is released into the creek at Station 7.2. At Station 8.7 (8.7 miles above the confluence with Todd's Ford), very little effect of pollution is noticed; the only sources of pollution are septic tank overflow and other runoff from farm lands and houses. Station 7.6 is somewhat polluted because of a break in the main leading from the city to the treatment plant. Below Station 7.2 the various sampling points were selected as indicative of the gradual return of the stream to clean conditions, and the various stations have been sampled for all types of stream life in order to describe this situation more completely.

The fact that a number of species of fungi are able to survive under conditions of extreme pollution and even dilution of this pollution indicates a major adjustment in their life requirements. It further indicates that they are probably aiding in the purification of the stream. If they are aiding in stream purification by using as food some of the various types of pollutant materials, this may indicate that they are both increasing the size of their thallus and producing spores. The production of spores in situations like this, which are subject to occasional tapping for irrigation water at least in some parts of the country, indicates that here is a potential source of inoculum for plant disease and pest species. In many cases this source need be present only for a limited period of time.

Nine of the 16 sampling points studied in the Dayton, Ohio, sewage treatment plant are listed in Table 3. These nine were chosen because they are locations where the largest buildup of fungus populations occurs or because they can be of importance as sources of fungus contamination through use as fertilizer or soil conditioner. While not listed, various effluents can be of some, although lesser, importance as indicated by Becker and Shaw (1955).

During the sampling period the flow chart at Dayton was similar to that of many secondary-type sewage treatment plants using trickling filters. Sewage entered the treatment plant through the detritus tanks in which large particulate matter is screened out. From here it flowed to Imhoff tanks for primary settling. The liquid flowed to the trickling filter dosing tanks, from which it was routed to either the high rate or standard rate filters. These filters are cement tanks 165 feet in diameter and 7.5 feet deep. They are filled with slag rock 1.5-3 inches in diameter, on which slime has developed including such organisms as bacteria, fungi, algae, and protozoans. The liquid sewage passes over this stone and out of the filters into a final settling basin and thence to the Great Miami River. Sludge in the final settling basin was returned to the Imhoff tanks to repeat the cycle. Sludge from the Imhoff tanks was passed to secondary digesters and then to drying beds where it was air-dried, removed, ground up, passed through a heated oven, pulverized, and sacked for sale as an organic soil conditioner.

In certain communities, sewages in various stages of treatment are used as sources of irrigation supplements. The raw sewage used can contain fungal spores introduced from such source as runoff from soils following precipitation or lawn and flower bed irrigation; washings from infected or moldy plant parts, mildewed materials, etc.; or contaminated plumbing where growth can occur between intermittent flow periods. In other communities, effluents from primary settling tanks or Imhoff tanks are used; and in some cases, secondary treatment processes may be by-passed. In still other communities, effluents from complete or secondary plants may be employed. Depending on the type of treatment, that is, whether recirculation is practiced or whether the plant is overloaded, the effluent may be rich or poor in fungus disseminules. There may be seasonal activity within the treatment plant or in the various places from which its populations are derived, which would give a seasonal pattern to the microorganisms in the effluent. The sludges used for supplemental fertilizers or soil conditioners may have developed a population of organisms that could produce trouble in the field or at least add to the populations of microorganisms in the field awaiting favorable conditions for infecting crop plants.

The paradox of this situation is that under certain circumstances a fungus that could cause considerable damage in a field could be actively purifying the sewage in which it is living. The test in which it was found that a strain of Fusarium oxysporum could reduce motor oil in dilution water is a case in point. Should a treatment for such wastes be developed in which this or similar fungi were the microbial agents, and should the effluents containing spores or other disseminules be applied as irrigation supplements indiscriminantly, it is likely that an inoculum for disease of a crop plant could develop and considerable economic loss result.

It thus appears that in these days of increased supplemental irrigation by use of sewage or polluted water the plant pathologist is presented with additional problems of disease control. Unfortunately, to control one fungus chemically may inhibit the work of many beneficial organisms, so that control measures necessary for irrigation waters should be applied to effluents rather than to influents of the sewage treatment plant. It is possible that adequate control of plant pathogens may also result in adequate control of human pathogens, so that a wider use could be made of sewage effluents than is being made today.

LITERATURE CITED

Becker, J. G., and C. G. Shaw, 1955. Fungi in domestic sewage-treatment plants. Applied Microbiology 3: 173-180.

Bush, A. F., and S. F. Mulford. 1954 Studies of "waste water reclamation and utilization". California State Water Pollution Control. Board 9; 1-82.

Cooke, W. B. 1954 (a,b,c). Fungi in polluted water and sewage.
I. Literature Survey. II. Isolation technique. III. Fungi in a small polluted stream. Sewage and Industrial Wastes. 26: 539-549, 661-674, 790-794.

Cooke, W. B. and P. W. Kabler. 1955. Isolation of potentially pathogenic fungi from polluted water and sewage. Public Health Reports. 70: 689-694.

Gaufin, A., and C. Tarzwell. 1952. Aquatic invertebrates as indicators of stream pollution. Public Health Reports. 67: 57-64.

Greenberg, A. E., and J. F. Thomas. 1954. Sewage effluent reclamation for industrial and agricultural use. Sewage and Industrial Wastes. 70: 689-694.

Henry, C. D., R. E. Moldenhauer, L. E. Englebert, and E. Truog. 1954. Sewage effluent disposal through crop irrigation. Sewage and Industrial Wastes. 26: 123-133.

Hunt, H. J. 1954. Supplemental irrigation with treated sewage. Sewage and Industrial Wastes. 26: 250-260.

Hutchins, W. A. 1939. Sewage irrigation as practiced in the Western States. U. S. Dept. Agr. Tech. Bull. 675: 1-59.

Ludzack, F. J., and D. Kinkead. 1955. The persistence of the motor oil class of hydrocarbons in polluted water under aerobic conditions. Mimeographed report. Robert A. Taft Sanitary Engineering Center. April 4. pp. 1-17.

Menzies, J. D. 1954. Plant disease observations in the irrigated areas of central Washington during 1953. Plant Dis. Reptr. 38: 314-5.

Merz, R. C. 1955. A survey of direct utilization of waste waters. California State Water Pollution Control Board 12: 1-80.

Schwab, C. E. 1955. Pollution-- a growing problem of a growing nation. Water. the Yearbook of Agriculture. pp. 636-643.

Skulte, B. P. 1956. Irrigation with sewage effluents. Sewage and Industrial Wastes. 28: 36-43.

Stone, R. 1956. Irrigation with waste waters. Public Works 86 (11): 97-98, 134-135.

United States Dept. of Agriculture. 1953. Plant Disease. The Yearbook of Agriculture. xviii & 940 pp. Washington. The United States Government Printing Office.

United States Dept. of Agriculture. 1955. Water. The Yearbook of Agriculture. xiii & 751 pp. Washington. The United States Government Printing Office.

ROBERT A. TAFT SANITARY ENGINEERING CENTER, 4676 COLUMBIA PARKWAY, CINCINNATI 26, OHIO.

Reproduced With Permission From:
JOURNAL OF THE NEW ENGLAND
WATER WORKS ASSOCIATION
75(1961): 37-50

WATER-BORNE TYPHOID EPIDEMIC AT KEENE, NEW HAMPSHIRE

William A. Healy[*] and Richard P. Grossman[†]

As an introduction to this discussion of the Keene typhoid epidemic, which occured in the later part of 1959, a brief description of the character of the watershed areas and the quality of supply will be of interest. The City of Keene was one of the first New Hampshire communities to undertake the construction of a public water supply system. Records on the subject indicate that in 1867, the sum of $82,000 was voted to purchase lands surrounding Goose Pond (42 acres), later called Sylvan Lake, and to install the necessary supply main and distribution lines in the central part of the City.

Goose Pond is about three miles north of the City, has a drainage area of 1.84 square miles and a safe yield of 1.08 m.g.d. The watershed is largely wooded, completely uninhabited and the shoreline is entirely controlled by the municipality. No recreational use is permitted and the pond is visited regularly for observation as to compliance with the sanitary restrictions in force.

Analytical records for this source of supply demonstrate that the bacterial quality of the raw water is excellent and falls well within the recommended M.P.N. limit of 50 coliforms per 100 ml. for acceptance as a drinking water supply after chlorination. Originally flow to the City was by means of gravity but Goose Pond water, which is now on a standby emergency basis, is delivered to the system by means of a 1,000 g.p.m. centrifugal pump, manually operated, located in a booster station approximately one mile downstream of Goose Pond. Simple chlorination is provided by a vacuum type chlorinator which operates whenever the pump starts.

Due to continued population and industrial growth, the City in 1886 turned to Woodward Pond (Echo Lake) in the neighboring towns of Roxbury and Nelson for additional supply. This body of water (104 acres) is located about five and one-half mile east of the City proper, and the natural lake level was increased ten feet by the construction of a dam. An intake structure, Quarry Dam, was developed about three miles below on the outlet stream, Roaring Brook and water was delivered by gravity to the one-million-gallon Octagon Reservoir on Beech Hill through three miles of 8-in. pipe. Later, in 1902, Stone Dam was constructed, one and one-quarter miles below Quarry Dam, impounding two million gallons, and a 12-in. supply main was laid through Marlboro Street into South Keene, connecting to the southern end of the distribution system (see Figure 1).

These facilities constituted the source of supply for several years and it might be well to note that as in the case of the Sylvan Lake development, the City did acquire considerable amounts of land in the watershed area for control and protective purposes. Despite such measures the Woodward Pond supply was essentially an untreated stream system and as such, more or less variable as to bacterial quality depending upon run-off conditions. Accordingly, it is not surprising that an epidemic of gastro-enteritis, with some 500 to 1,000 cases, involving the public water supply as the transmission agent, occurred in October, 1923. Following this incident, slow sand filters were installed at Beech Hill and Stone Dam. A 1.5 m.g. reservoir was also included in the Beech Hill project. The Beech Hill plant capacity was rated at 750,000 g.p.d., at 5 m.g.d. per acre, and is operated at 0.9 m.g.d. At Stone Dam 1.5 m.g.d. capacity, at 5 m.g.d. per acre, was provided, which later was increased to 3.0 m.g.d. by the construction of two additional filters in 1952. These filters are operated at 1.1 m.g.d. due to limitations in transmission capacity. In 1931 Babbidge Reservoir with a capacity of 160,000,000 gallons was developed about one-quarter of a mile above Stone Dam.

Except for certain improvements in intake pipe capacities and the development of a gravel-walled well (the bacteriological record of which is excellent) in West Keene, the supply for the City remained in this stage up to the time of the typhoid epidemic in October-November, 1959. In passing it might be of interest to observe that the watershed area of the Roaring Brook Source amounts to 4.9 square miles and the safe yield is 2.55 m.g.d. There are only a few dwellings on the watershed, a limited number of roads, most of which are little used, and some of which are impassable. In essence then we have a remote, lightly inhabited watershed, mostly wooded and one obviously capable of producing a supply safe

[*] Director and [†] Senior Engineer, Division of Sanitary Engineering, New Hampshire Department of Health, Concord, N.H.

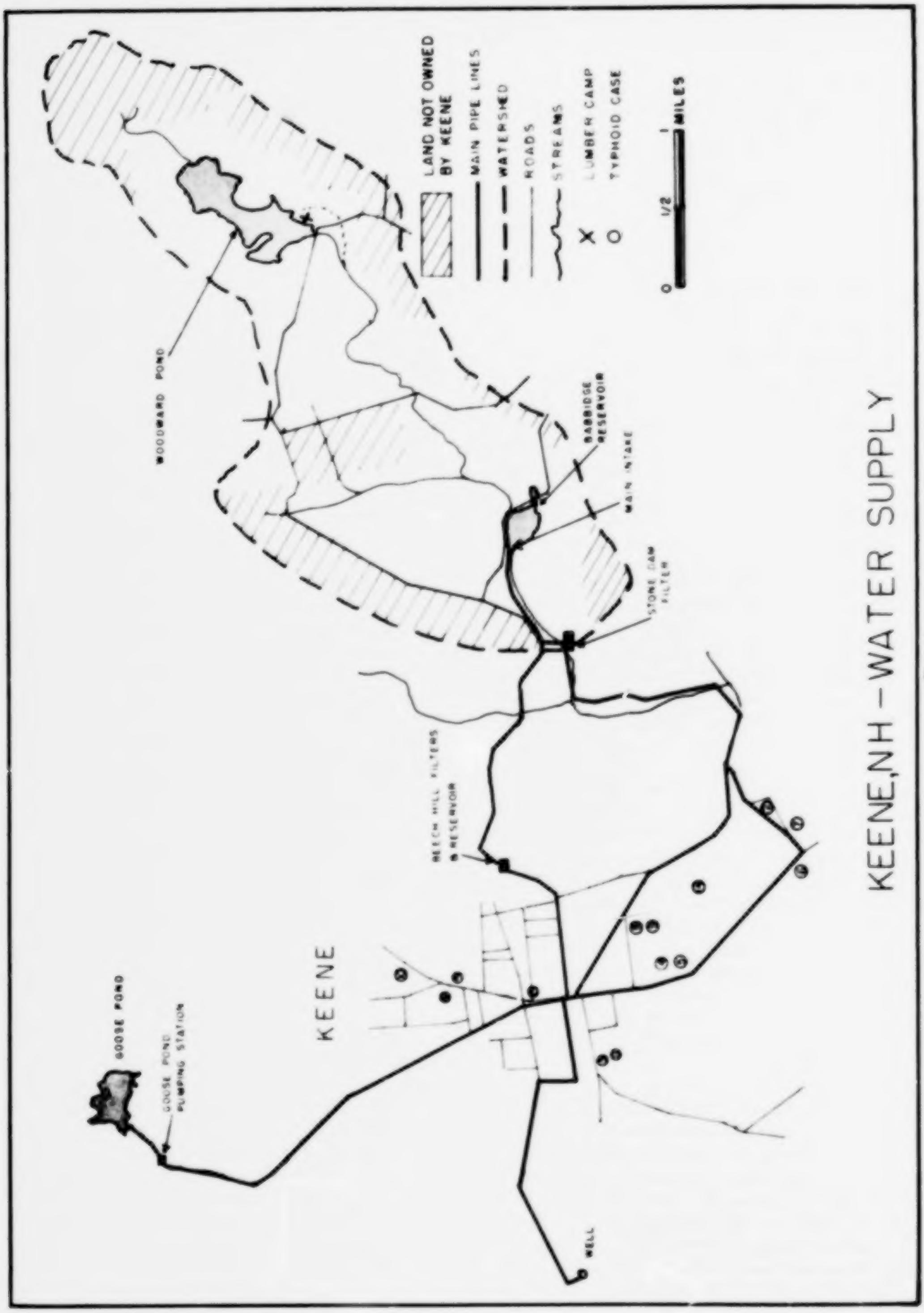

KEENE,NH - WATER SUPPLY
KEENE
GOOSE POND
GOOSE POND PUMPING STATION
WELL
BEECH HILL FILTERS & RESERVOIR
WOODWARD POND
BABBIDGE RESERVOIR
MAIN INTAKE
STONE DAM FILTER
LAND NOT OWNED BY KEENE
MAIN PIPE LINES
WATERSHED
ROADS
STREAMS
X LUMBER CAMP
O TYPHOID CASE
0
1/2
1
MILES

Fig. 1

for drinking with a minimum in the way of treatment. A review of the analytical records on raw water samples submitted to the state and local health department laboratories from the Roaring Brook supply, during the period since July, 1956, to the time immediately prior to the typhoid outbreak, disclosed that the quality conforms to the U. S. Public Health Service "Manual of Recommended Water-Sanitation Practice" (1946) for surface water supplies to be subjected to simple chlorination, which requires that the average Most Probable Number of coliform organisms in any one month shall not exceed 50 per 100 ml. A composite of these results appears in Table 1 and a similar tabulation for distribution-system quality is presented in Table 2. It is of interest to note from the tables that raw water quality meets the recommended practice throughout the period of tabulation except for the month of October, 1959, at which time the epidemic started.

The U. S. Public Health Service Drinking Water Standards require that:

(1) Of all the standard ten milliliter portions examined per month, in accordance with the specified procedure, not more than ten percent shall show the presence of organisms of the coliform group.

(2) Occasionally three or more of the five equal ten milliliter portions, constituting a single standard sample, may show the presence of organisms of the coliform group, provided that this shall not be allowable if it occurs in consecutive samples or in more than:

  (a) Five percent of the standard samples when twenty or more samples have been examined per month.

  (b) One standard sample when less than twenty samples have been examined per month.

An examination of Table 2 reveals that distribution-system water quality failed to meet these standards on some occasions and, while generally good quality is indicated during the period of record, it is of significance that October, 1959, is included as one of the months of failure to satisfy the standards.

Experience with slow sand filters suggests that the percent efficiency in the removal of bacteria which can be expected is in the range of 98 to 99 percent. However, at Keene the rate of operation at the Beech Hill filters has been appreciably above the conventional loading of 2.5 m.g.d. per acre, and this would tend to decrease the efficiency of removal. Much more important, however, is the problem of greatly lowered efficiency immediately following the cleaning of filters and this inherent characteristic is readily discernible from an examination of laboratory data collected during the typhoid incident. Also a study of the routine analyses of distribution system samples will show a very close correlation with water plant records as to times of filter sand cleaning. This deficiency caused the State Department of Health and the City's Consultant, Camp, Dresser and McKee, to recommend several years ago the regular use of chlorination for disinfection purposes.

However, in view of the generally good conditions prevailing in the watershed, the absence of water-borne illnesses, confidence in the existing filtration facilities and in particular, a strong aversion to a chlorinated supply, the City government rejected these recommendations.

October, 1959, was a month of heavy rainfall with especially heavy precipitation being experienced on the dates of October 24 and 25. As will be noted from Table 3, the rainfall for October 25th alone was 1.3 times the monthly average and this coupled with the previous day's rainfall resulted in a situation of extremely high runoff in the watershed area. That this condition obtained is readily evident when we examine the laboratory results for this portion of the month (Table 4). Clearly, any pollutional material present in the upper reaches of the drainage basin would quickly be transported down into the system and later investigation established that this is precisely what happened. On Friday, October 23, just preceding the heavy rainfall of the 24th, one of the four slow sand filter units at Stone Dam was cleaned and placed in service shortly thereafter, in accordance with regular routine. Referring again to Table 4, the records of distribution-system sample collections for the dates of October 26, and immediately following, showed evidence of substantial numbers of coliform organisms. Also raw water collections from Babbidge Dam for the same period, indicated coliform counts appreciably higher than normal, however, apparently no great significance was attached to these findings at the time. As a matter of fact, it will be recalled that elevated counts in the distribution system were normally observed after filter cleaning procedures and it is probable that these were accepted on that basis. In any event, on November 6, the first patient, a six-year-old boy, was admitted to the Elliot Community Hospital in Keene, suffering from severe and recurring nose-bleeds. High fever, nausea and vomiting were also present. Subsequently, between November 10 and November 14, four more patients were hospitalized, all running high fevers with other symptoms ranging from headache and general malaise to chest and abdominal difficulties. Preliminary agglutination studies were performed on these patients and the results were such that the physicians involved were strongly suspicious that they were dealing with typhoid fever. During the next several days the remaining nine of the fourteen recognized cases of typhoid developed and by subsequent questioning, it became apparent that all began to experience their symptoms between November 6 and 19.

On November 22, the Keene Health Department notified Dr. Edward W. Colby, State Health Officer, of the presumptive diagnosis of typhoid fever and requested assistance of the State Department of Health. From that time on, the investigation was performed

TABLE 1—BACTERIOLOGICAL RESULTS—KEENE, N. H., WATER SUPPLY RAW WATER
July, 1956, to July, 1960

Date	No. of samples	No. positive samples	MPN/100 ml.		Date	No. of samples	No. positive samples	MPN/100 ml.	
			Range	Average				Range	Average
1956					*1958*				
July	16	8	2.2-15	5.0	July	11	8	2.-2-5.0	2.9
Aug.	16	8	2.2-38	9.1	Aug.	15	2	2.2	2.2
Sept.	11	9	2.2-15	6.0	Sept.	13	3	5.0-15	9.6
Oct.	13	1	5.0	5.0	Oct.	15	6	2.2-240	82.4
Nov.	12	0	—	—	Nov.	10	1	2.2	2.2
Dec.	13	2	8.8-15	11.9	Dec.	16	1	2.2	2.2
1957					*1959*				
Jan.	12	3	2.2	2.2	Jan.	13	0	—	—
Feb.	9	0	—	—	Feb.	7	0	—	—
Mar.	12	0	—	—	Mar.	13	0	—	—
Apr.	12	0	—	—	Apr.	14	2	2.2-5.0	3.6
May	16	1	2.2	2.2	May	11	0	—	—
June	12	5	2.2-38	9.9	June	13	4	2.2-8.8	5.2
July	16	10	2.2->240	?	July	13	6	2.2-240	44.9
Aug.	10	3	2.2-5.0	3.1	Aug.	14	8	2.2-15	4.5
Sept.	14	6	2.2-15	4.8	Sept.	11	8	2.2-8.8	4.2
Oct.	15	3	2.2-8.8	5.3	Oct.	12	10	2.2-240	73.5
Nov.	11	0	—	—	Nov.	11	9	2.2-15	3.9
Dec.	12	5	2.2-240	52.8	Dec.	12	4	2.2-240	61.6
1958					*1960*				
Jan.	12	3	2.2-8.8	6.6	Jan.	14	0	—	—
Feb.	11	0	—	—	Feb.	12	0	—	—
Mar.	15	0	—	—	Mar.	12	0	—	—
Apr.	11	0	—	—	Apr.	13	0	—	—
May	12	1	2.2	2.2	May	12	0	—	—
June	13	3	—	16.3	June	15	0	—	—

TABLE 2—BACTERIOLOGICAL RESULTS—KEENE, NEW HAMPSHIRE, WATER SUPPLY DISTRIBUTION SYSTEM

July, 1956, to July, 1960

Date	No. of samples	No. positive samples	MPN/100 ml. range	% 10-ml. tubes positive	Date	No. of samples	No. positive samples	MPN/100 ml. range	% 10-ml. tubes positive
1956					*1958*				
July	42	7	2.2-8.0	4.8	July	42	8	2.2	3.8
Aug.	44	5	2.2-8.8	3.7	Aug.	42	0	—	0
Sept.	46	7	2.2-8.8	4.8	Sept.	42	2	2.2	1.0
Oct.	42	2	5.0	1.9	Oct.	46	2	2.2-15	2.3
Nov.	40	0	—	0	Nov.	28	2	2.2	1.4
Dec.	36	3	2.2	1.7	Dec.	46	1	2.2	0.4
1957					*1959*				
Jan.	41	3	2.2-240	3.5	Jan.	38	1	2.2	0.5
Feb.	36	0	—	0	Feb.	26	0	—	0
Mar.	38	0	—	0	Mar.	45	0	—	0
Apr.	40	1	38	2.5	Apr.	43	0	—	0
May	45	1	2.2	0.5	May	38	0	—	0
June	34	7	2.2-15	6.5	June	39	1	38	2.6
July	48	9	2.2	3.7	July	42	10	2.2-5.0	5.2
Aug.	38	4	2.2-7.6	3.2	Aug.	42	13	2.2-240	14.8
Sept.	40	7	2.2-21	6.0	Sept.	40	16	2.2-5.0	9.0
Oct.	48	1	2.2	0.4	Oct.	42	12	2.2-240	11.9
Nov.	36	0	—	0	Nov.	36	10	2.2-8.8	6.3
Dec.	38	6	2.2	3.2	Dec.	46	5	2.2-8.8	3.0
1958					*1960*				
Jan.	38	2	2.2	1.1	Jan.	42	0	—	0
Feb.	36	0	—	0	Feb.	40	0	—	0
Mar.	44	1	5.0	0.9	Mar.	46	0	—	0
Apr.	35	2	2.2-5.0	1.7	Apr.	41	1	5.0	1.0
May	42	2	2.2-15	2.4	May	42	1	5.0	1.0
June	42	1	2.2	0.5	June	46	2	2.2-38	2.6

TABLE 3—PRECIPITATION FOR THE MONTH OF OCTOBER, 1959, AT KEENE, NEW HAMPSHIRE

Date	Rainfall in inches
October 1	.08
2	.52
6	.52
7	.53
8	.45
9	.56
10	.13
12	.20
14	.01
18	.16
19	.07
21	.02
23	.28
24	.73
25	3.56
27	.02
31	trace
Total	7.84

Mean rainfall for month of October—2.7 inches

TABLE 4—BACTERIOLOGICAL RESULTS—KEENE, N. H., WATER SUPPLY—RAW AND DISTRIBUTION SYSTEM

October 26 to November 12, 1959

Date	MPN/100 ml. in raw water	MPN/100 ml. in distribution system	
October 26	240		
27		38	5.0
28	240	38	15
29		2.2	
30	38		
November 2	15	2.2	8.8
3		2.2	2.2
5		2.2	2.2
6	5.0	< 2.2	< 2.2
10		< 2.2	2.2
12	2.2	< 2.2	< 2.2

jointly by State and local authorities and the Epidemic Intelligence Service of the U. S. Public Health Service.

The patients were interviewed extensively regarding the onset of illness, source of drinking water supply, milk supply, food supply, place of work, living conditions, previous family history of typhoid illness, etc. Literally every possible avenue which would disclose a common source of infection was explored inasmuch as the pattern of onset of illness suggested a single source of infection. Further, it was estimated that the contamination would have had to take place some time between October 25 and November 2, taking into account the normal period of incubation for typhoid. The earlier date seemed more probable since one of the patients was found to have been out-of-state on October 31 and November 1. This assumption becomes a certainty when viewed in the light of the results of water examinations performed on samples taken on October 26.

The results of the field studies, conducted up to November 29, concerning the origin of infection are summarized in Dr. Alvin Novack's (U. S. Public Health Service) report, from which the following is quoted:

"1. Geographically, there was no direct relationship between patients.

2. Socially there was little or no association.

3. Only one or two families had been to any restaurant in the preceding six weeks.

4. The families were supplied by four different milk companies.

5. The families dealt with nine different food stores. Three or more families patronized the same store in four instances. Five families obtained their food from the same food store, the highest degree of correlation.

6. There were no known Salmonella Group D carriers in the area.

7. The only common denominator for all patients was the city water supply."

About this time reports were received concerning a water main break having occured in the southern section of the distribution system during the latter part of October. Also, that the West Street gravel-packed well had been flooded and finally that there was a logging operation going on in the upper end of the Woodward Pond watershed. All three conditions were investigated with the first two being found to have no connection with the problem. The inspections made at the lumbering operation on November 30 and December 1 were very revealing. The logging camp was occupied by three people and was located on a slope some 200 to 300 yards distant from the pond but draining away from it. Situated nearby was the barn housing two horses and all drainage took place toward a small intermittent brook about 125 feet away.

It was believed that the stream flow took place off the watershed but traveling along the course of the brook, it was found that final discharge was to Roaring Brook some quarter of a mile downstream of the pond. At the rear of the barn was an accumulation of five to six feet of horse and human excreta. There were no toilet facilities whatever and disposal of all wastes was to the manure pile. The three people at the camp were taken to the hospital for blood and stool examinations, the camp removed, the barn burned and the wastes completely removed from the area, after which the ground was liberally treated with chloride of lime. The stool culture from one of the men was shown to contain Salmonella group D organisms and he was presumably responsible for the outbreak. Meanwhile, chlorination of the supply was ordered, and installation was undertaken on December 2. Presently Stone Dam and Beech Hill water sources are being chlorinated separately. The permanent installation now in process of design will provide for a single plant on the supply main upstream of the point at which the line branches to the two filter plants.

To return to the matter of organism identification, examination by the local hospital laboratory disclosed the presence of Salmonella Group D cultures from either or both blood and stool specimens of all fourteen typhoid patients. These results were confirmed in the diagnostic laboratory at the State Hospital, Concord. For all practical purposes then, this information coupled with the data concerning the carrier employed in the lumbering operation, the extreme rainfall washing fecal material into the supply, the bacteriological results of water sampling during the period (see Table 4), the sudden onset of the disease and the timing of the filter cleaning were sufficient to demonstrate that the epidemic was caused by the public water supply. There remained one significant refinement or final step which would complete the epidemiological study, and this involved the phage typing of cultures. Phage typing is a laboratory method whereby the individual strains of organisms within a single group may be specifically identified by means of a series of special agglutination reactions. These tests were conducted at the Enteric Bacteriology Unit, Communicable Disease Center, Chamblee, Georgia, and from this work it was apparent that all patients had been infected with an organism which is designated as Salmonella typhi Phage Type E. The same organism was recovered from the lumberjack carrier, which serves to complete the cycle and conclusively establish the common association between the fourteen typhoid cases and the carrier. So far as we know, this is the first time it has been possible to utilize an advanced technique of this type in tracing a water-borne outbreak of typhoid fever. Clearly, it has been an invaluable link in establishing the chain of evidence at Keene, even to the extent that the investigation may come to be regarded as a classic demonstration of the epidemiology of water-borne typhoid.

An interesting or unusual sidelight to the problem is the fact that relatively few cases were experienced. According to the U. S. Public Health Service authorities, it would be reasonable to have expected upwards of 180 to 250 cases of typhoid with the water supply as the vehicle of transmission. In our opinion, the fact that no such high incidence of cases occured may be explained through the extremely high dilution which the rainfall of October 24 and 25 afforded. Secondly, the filtration facilities, although not operating at normal efficiency, did provide another barrier to the spread of the infection, and these two factors could readily account for the small number of cases. As concerns the final outcome of the incident, thirteen of the typhoid patients recovered from the illness and one death resulted because of other complications. A regular schedule of stool examinations is being maintained in order to evaluate the possible carrier problem. Developments from this study are gratifying inasmuch as the findings at the present time are all running negative. There is no practical method for determining whether or not subclinical cases occured and, if so, whether carriers will develop from this source. Only time will provide an answer to this question.

Now as to what this unfortunate episode has taught us. One obvious thing is that the threat of intestinal water-borne disease is still very much a reality despite the general excellence of water supply quality which has been established over the years through the progressive efforts of water supply management, engineers and health authorities. Here we have an example where a rare set of circumstances were required to produce illness of epidemic proportions, namely, the carrier, torrential rains and a filter plant failure. The rarity of occurrence is verified by the fact that this lumbering situation existed on the watershed for well over a year without prior indicent. Looking at the problem in a slightly different way, we can say with ample justification that there is still a real necessity for maintaining stringent watershed controls which has been the practice in New England and other regions of the country for a great many years. All of us have witnessed or participated in discussions where great emphasis is placed, by those who seek to open up water supplies to recreational or other use, upon the absence of disease and the "negligible" risk involved when multiple uses are permitted. The unhappy consequences of this approach is that we begin to stray immediately from the basic concept and purpose which prompted restrictions on use and a policy of controlled conditions in the first instance. Water-supply management has the fundamental responsibility of producing and maintaining a safe supply and, in view of this obligation, has no alternative to a carefully planned operation which is aimed at prevention. Health agencies operate within the same realm of responsibility and it should not be our purpose to provide a cure where there is an opportunity to prevent. In the final analysis, we in the water supply field are striving to come as close to 100% protection for the consumer as is reasonably possible of accomplishment.

With the knowledge that personnel, though conscientious, remain subject to error, and facilities are likewise capable of failure or inadequacy, it would seem that the only logical solution to the question of watershed use lies in the policy expressed by the American Water Works Association* and essentially concurred in by the New England Water Works Association, † whereby recreational or other use of water bodies used directly as a source of drinking-water supply is rejected as an unsafe practice.

ACKNOWLEDGMENT

The epidemiological investigations and studies of the typhoid epidemic at Keene, New Hampshire, in November, 1959, were undertaken jointly by the City and State Health Departments and the U. S. Public Health Service.

City of Keene officials participating in the work were:

Joseph R. Bruce, Superintendent of Water Works Department
Joseph F. Burke, Superintendent of Public Works Department
Robert G. Shaw, City Engineer
Dr. Thomas Lacey, Chairman Keene Board of Health
Evan C. White, Health Officer
Donald E. Chick, City Manager

Actively associated with the studies from the State level were:

Dr. Edward W. Colby, State Health Officer
Dr. William M. Prince, Director, Bureau of Communicable Disease Control
Robert Miliner, Supervisor, Clinical Laboratory, N. H. State Hospital
Staff personnel from Division of Sanitary Engineering

We further wish to acknowledge the valuable work of Dr. Alvin Novack, Epidemic Intelligence Service Officer, U. S. Public Health Service, Atlanta, Georgia.

In the preparation of this paper, free use was made of reports, correspondence and material resulting from conferences held with the above-named officials. The authors gratefully acknowledge their unselfish cooperation and assistance which made the preparation of this paper possible.

---

*Jour. A.W.W.A., vol. 50, May, 1958, p. 580.

†Jour. N.E.W.W.A., vol. 72, no. 4, Dec., 1958, p. 355.

Chapter VI

BIOLOGY OF POTABLE WATER SUPPLIES

Reproduced With Permission From, and Copyrighted By:
JOURNAL OF THE AMERICAN
WATER WORKS ASSOCIATION
37(1945): 963-984

TRANSFORMATIONS OF IRON BY BACTERIA IN WATER

Robert L. Starkey

Assoc. Microbiologist, New Jersey Agric. Expt. Sta.,
Rutgers Univ., New Brunswick, N.J.

Conditions in water favor the growth of many different microorganisms. Principal among these are algae which require only mineral nutrients from the water and obtain their energy from sunlight. There are also many bacteria, as well as filamentous fungi and protozoa, that grow on organic materials and develop extensively in waters containing large amounts of organic matter. Still other bacteria can develop in water containing little or no organic matter but in which there are ferrous or manganous compounds, sulfides or certain other incompletely oxidized mineral substances which can be used by iron bacteria, sulfur bacteria or other autotrophic bacteria as sources of energy for growth. The development of algae, heterotrophic microorganisms and autotrophic bacteria may be so profuse in water supplies that it is necessary to provide a means to prevent their growth and the formation of their objectionable products. In developing methods of control it is desirable to establish the nature of the organisms causing the trouble and to know the characteristics and growth requirements of these organisms.

The iron bacteria, which are members of the third group, are some of the most important fouling organisms, since they not only produce troublesome accumulations of cell material but also still greater quantities of ferric hydrate. In addition to the iron bacteria there are various other bacteria, including sulfur bacteria and sulfate-reducing bacteria, which are responsible for various transformations of iron. Some bring iron into solution, others cause its precipitation and, in certain instances, the bacteria are responsible for corrosion. These reactions may be due to changes in pH, to oxidation or reduction, to production of sulfide, to differences in concentration of CO_2 or to the formation and decomposition of organic compounds of iron, all of which can be brought about by microorganisms. The reactions affect inorganic compounds of iron in solution or in the solid state, organic compounds, and even metallic iron.

Some of the iron transformations are brought about by bacteria having specific physiological characteristics intimately associated with the iron transformations, whereas in other cases the transformation of the iron is incidental to the normal development of the bacteria. It is important to establish the characteristics of the different bacteria in order to understand their relationships to various changes which iron undergoes. Some of these bacteria and their actions are:

1. Iron bacteria. Oxidize ferrous iron to ferric iron and effect the precipitation of ferric hydrate.

2. Non-specific bacteria. Cause changes in the iron content of water by altering the environmental conditions.

   (a) Cause changes in reaction. Increase in acidity tends to bring iron into solution while decrease in acidity tends to cause precipitation.

   (b) Cause changes in the degree of oxidation and reduction. Oxidation tends to favor precipitation, whereas reduction favors solution.

   (c) Produce and decompose organic compounds of iron. Organic iron compounds which are slightly ionized tend to remain in solution but decomposition of slightly ionized organic compounds of iron leads to iron precipitation.

   (d) Cause changes in the CO_2 content of water. Increase in CO_2 content tends to favor solution, large amounts of CO_2 may cause precipitation, and decrease in CO_2 content favors precipitation.

3. Sulfur bacteria. Some of the bacteria increase the acidity and thus tend to dissolve iron; under specific conditions insoluble iron sulfate may be produced.

4. Sulfate-reducing bacteria. Iron becomes precipitated by a direct reaction with sulfide which is produced by sulfate reduction. These bacteria also effect the anaerobic corrosion of iron, but by another reaction.

It is apparent that there are numerous changes of iron in which bacteria take part in addition to those which are brought about by the iron bacteria. Whereas non-specific bacteria may be associated with iron precipitation, some of them are also important as agents of solution of iron, and, as such, provide conditions favorable for subsequent precipitation which may lead to turbidity of water or fouling of pipes.

IRON BACTERIA

The development of iron bacteria may manifest itself in several ways. There may be hard deposits which tend to fill up the pipes and reduce their water-carrying capacity. Slime and accumulations of filamentous bacteria may form on the walls of pipes and water holders and scum may be produced on the water surface. These bacteria may cause turbidity and discoloration of water and be responsible for some of the unpleasant tastes and odors which are produced either directly or indirectly as the dead bacterial cells are decomposed by other microorganisms Cases of very profuse development of the filamentous iron bacteria have been referred to as "water calamities."

Control problems have arisen with water from some deep wells (1, 3) and organic soils (8), since these waters carried considerable amounts of iron or manganese in solution as inorganic or organic compounds. Surface waters may also cause difficulties, due to the iron content of the raw water as well as to iron which becomes dissolved from pipes and other equipment. Larson (5) reports a case where the water was iron-free upon leaving the plant but where there was as much as 8 ppm iron at the end of the line a short distance away which gave rise to "red water." Tenny (8) reports a condition in Des Moines, Iowa, where water, collected below ground, showed an increase in iron content of more than 0.6 ppm. In the concrete gallery where the water was gathered iron bacteria formed thread-like growths coated with ferric hydrate, some of which broke loose and gave the water a red color. *Gallionella* was particularly prominent, but *Leptothrix* and *Sphaerotilus* were also encountered. Iron bacteria are believed by Carroll (3) to have been responsible for bad odor and taste of water which came from deep wells. There was a heavy deposit of soft brown material in the reservoir, and the water at the ends of the lines had a black color. Alexander (1) states that even though the water from deep wells may be free from bacteria and odors when the deposit is first tapped, bacteria and their products may appear after the deposit has been drawn upon for some time. The result is formation of slime and development of iron and sulfur bacteria in the tanks and pipes, causing the water to have an objectionable odor and taste.

The iron bacteria are typical water organisms. They are aerobic, and many of them differ morphologically from the common bacteria in that they are filamentous. They are widely distributed in nature and are found commonly in stagnant water, wells, mines, springs and in quiet parts of streams, marshes and lagoons, as well as in reservoirs and water pipes, but seldom in open water. They are not normal inhabitants of water that does not contain an appreciable amount of iron in solution.

They are characterized by an unusual capacity for accumulating ferric hydrate around their cells, which gives them a brown torust-red color. Manganese compounds also are oxidized by some of the iron bacteria and compounds of manganese may sometimes exceed in amount those of iron in the material precipitated by the bacteria (7, 11). It is generally assumed that the iron and manganese have played some role of importance to the cells preceding their precipitation.

The iron bacteria are fouling organisms and not agents of corrosion. It sometimes happens that the surface of the fouled metal shows evidences of corrosion, but in such cases it is probably that the corrosion was caused only indirectly by the iron bacteria.

Iron bacteria are particularly important fouling organisms since they accumulate large amounts of ferric hydrate, amounts many times greater than those of the bacterial cell material. It has been calculated that, as a result of growth of the bacteria on the ferrous iron in solution, there will be about 500 times as much precipitate of ferric hydrate as cells of the iron bacteria. This is of particular importance in establishing the cause of water troubles. It is apparent that, in cases where the iron bacteria have been the sole cause of the trouble, there will be an abundance of ferric hydrate and that the cells of the bacteria will be comparatively inconspicuous. If the fouling material consists principally of bacterial filaments and slimes with comparatively little ferric hydrate, the condition could not have been due to growth of the bacteria on ferrous iron to any appreciable extent. In such cases it is more likely that the bacteria had developed on the organic constituents of the water or some material other than the ferrous iron.

Some of the ferric hydrate which becomes precipitated on the walls of a pipe may come from the pipe itself and go into solution at one point only to be oxidized and deposited elsewhere. The principal source of iron in many cases, however, is the water delivered to the distribution system. This has been demonstrated where deposition has been controlled by freeing water of iron at the water works. There is further evidence in the results of Schorler (7), who found that pipes which were coated inside with asphalt became heavily incrusted with iron even though the surface of the pipe was intact and free from corrosion. Iron bacteria also develop on concrete if the water contains sufficient iron and if other conditions are favorable for their development. A case of this type was discussed by Tenny (8). Reddick and Linderman (6) concluded, however, that cement prevents tuberculation, since it both covers the metal and makes the reaction alkaline.

Further evidence that the iron need not come from the surface upon which the iron bacteria develop is provided by tests made with inert materials. In cases where iron deposition occurred, growth of iron bacteria was obtained on glass surfaces submerged in the water. In fact, microscope slides and cover-slips which have been exposed in this way have been used to study the morphology of iron bacteria.

Although it is important to microbiologists to establish the mechanism of precipitation, this information is not always necessary in order to deal with the troublesome accumulation of iron deposits in water distribution systems. Nevertheless, more definite information regarding these bacteria may serve as a guide for the development of more effective control measures. The pertinent fact is that these bacteria occur in accumulations of precipitated iron and are concerned in the formation of these accumulations. Any means which can be used to prevent their development can be expected to aid in preventing fouling. Control procedures may center about the destruction of the bacteria themselves or the removal of the iron from the water before it enters the distribution system. Their ability to precipitate iron depends on the composition of the water, and particularly on the presence of iron in the water and the compounds in which it occurs.

Many conflicting ideas have been expressed concerning the iron bacteria, their physiology and their relationships to the precipitation of iron. According to some investigators they are the principal agents of iron precipitation in nature and the cause of most of the trouble resulting from iron precipitation in water works and distribution systems. Others consider that these bacteria are but one of several agents responsible for the precipitation of iron. There is similar lack of agreement regarding the means by which the iron is precipitated by the bacteria. Whereas there is widespread belief that iron bacteria are autotrophic--that is, that they live on the energy released by the oxidation of ferrous iron to ferric iron--it is contended by others that many, if not most, of the iron bacteria live on organic materials and effect the precipitation of iron incidentally during their growth. It is probable that the iron bacteria are not all alike physiologically and that no single explanation of the precipitation of iron would be equally applicable to all of them. There is no reason to doubt, however, that there are bacteria which become coated with ferric hydrate, that these bacteria have distinctive morphological characteristics, or that their normal habitat is iron-bearing waters.

Morphology

The iron bacteria can be placed in three different morphological groups:

1. *Haplobacteria*, which are shaped like the common bacteria; and which occur singly or in irregular aggregates.

2. *Trichobacteria* or filamentous bacteria, whose filaments are composed of chains of cells enclosed in a sheath.

3. *Stalked bacteria*, which are oriented from top to base.

They have been classified more in detail under three orders in several different genera, the principal of which are listed below:

- Order I. *Eubacteriales* (true bacteria)
  - Genus 1. *Siderocapsa*
- Order II. *Chlamydobacteriales* (filamentous bacteria)
  - Family 1. *Chlamydobacteriaceae*
    - Genus 1. *Sphaerotilus*
    - Genus 2. *Clonothrix*
    - Genus 3. *Leptothrix*
    - Genus 4. *Crenothrix*
- Order III. *Caulobacteriales* (stalked bacteria)
  - Family 1. *Gallionellaceae*
    - Genus 1. *Gallionella*

Many additional genera of iron bacteria have been proposed and also numerous species of most of these genera have been described, but only a few typical species are mentioned below. (For further details see the monographs by Cholodny (13) and Dorff (15).

Siderocapsa. These organisms are coccoid cells or short rods occuring in small colonies composed of from one to 30 cells, most frequently less than ten, surrounded by a mucoid capsule. In all colonies the outer deposit surrounding the capsule of a single colony or a group of colonies is rust-brown in color due to accumulated ferric hydrate. The cells of *S. treubii* are 0.6 to 1.6 μ in diameter, whereas *S. major* is a small rod measure 1.4 to 1.0 x 2 μ. The cells are readily stained by an ammonium-oxalate crystal-violet solution. Most results seem to indicate that these bacteria live on organic materials and are not autotrophic. It is possible that the occurrence and development of *Siderocapsa* is sometimes overlooked and their importance as fouling organisms is underestimated, since there are less readily detected than the large filamentous bacteria.

Chlamydobacteriales. Bacteria of this order are filamentous, alga-like and sheathed. Most of them are unbranched, but some show false branching. In the latter case the branches arise following displacement of the cells in the filament from which new branches are formed. Thus the sheath is branched whereas the filaments are separate. The sheath may be composed entirely of organic matter, or of organic matter impregnated with iron, or entirely of ferric hydrate. In some cases conidia and motile swarmer cells are produced.

Sphaerotilus (Cladothrix). These are colorless filaments attached at the base and showing false branching. The filaments are composed of rod-shaped or oval cells surrounded by a firm sheath which is soft and slimy and contains no ferric hydrate. Reproduction is by non-motile conidia or motile swarmer cells. The most common species is *S. natans*. It is very widely distributed and is generally found in waters containing organic materials such as sewage or water where leaves and algae are undergoing decomposition. It has been cultivated in organic media, but its ability to live on iron compounds is questioned.

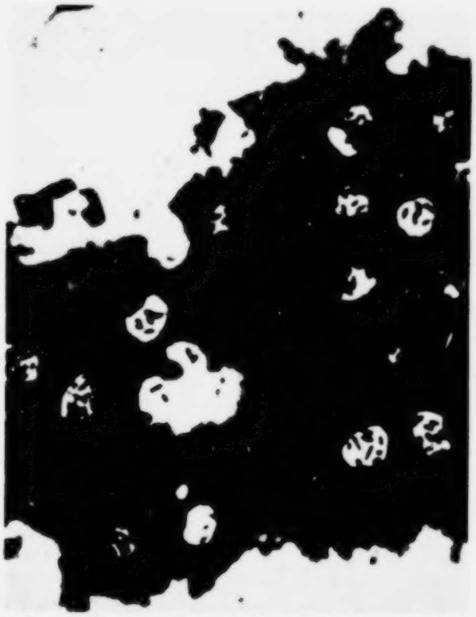


Figure 1 - Siderocapsa treubii. Multiple colonies surrounded by ferric hydrate. Mag. about 500X. From Hardman and Henrici (17), Fig. 4.

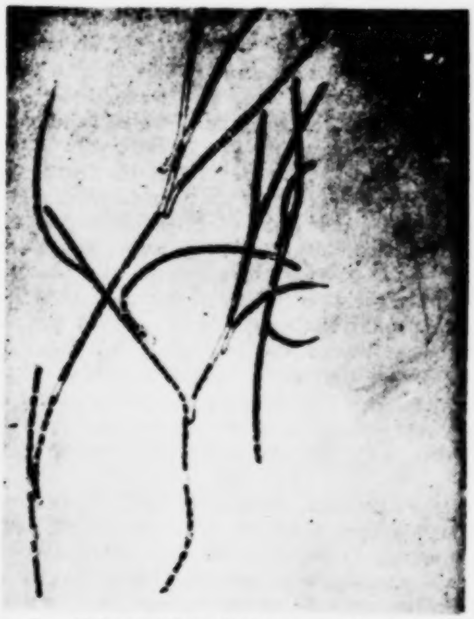


Figure 2 - Sphaerotilus dichotoma. Sketch showing false branching. Mag. about 230X. From Molisch (18), Fig. 3b.

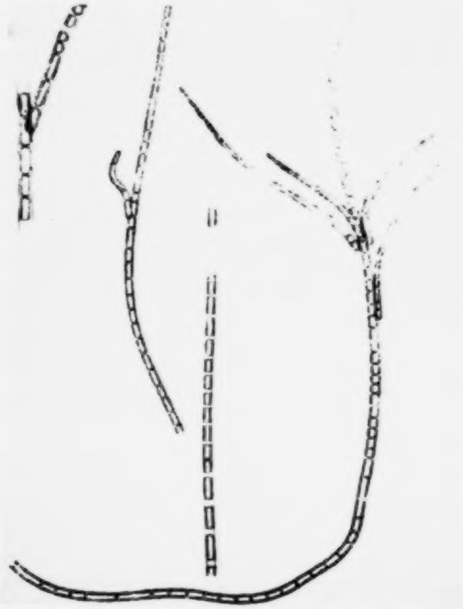


Figure 3 - Clonothris ferruginea. Sketch showing cells enclosed within a sheath, and false branching. Mag. about 430X. From Molisch (18), Fig. 4.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

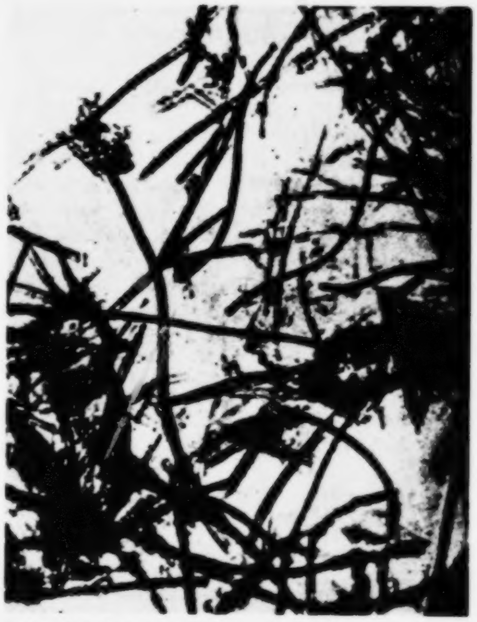


Figure 4 - Leptothrix ochracea. Sheaths from an accumulation of precipitated ferric hydrate in iron-bearing water. Mag. about 390X. From Molisch (18), Fig. 5.

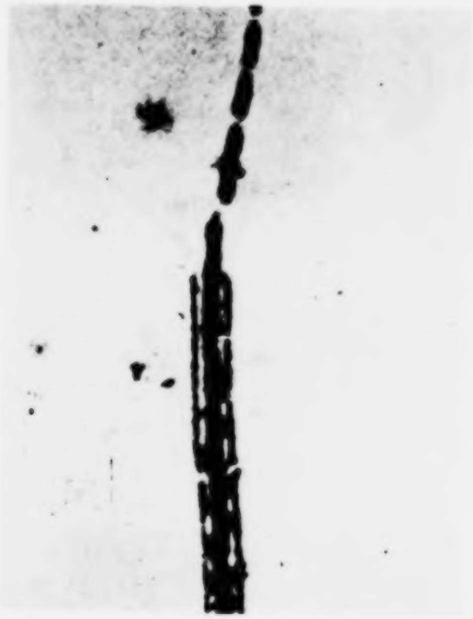


Figure 5 - Leptothrix ochracea. Cells coming out of their sheath. Mag. about 2200X. From Cholodny (13), Plate 4, Fig. 20.

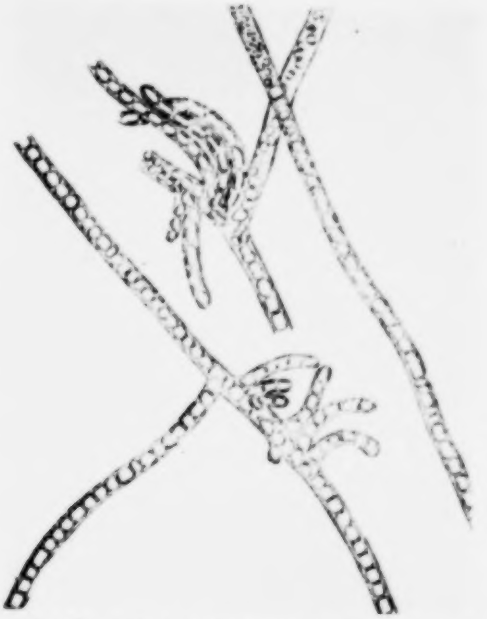


Figure 6 - Crenothrix polyspora. Sketch showing details of false branching of cells withing the sheath. Mag. about 380X. From Harder (16), Plate 1, Fig. A.

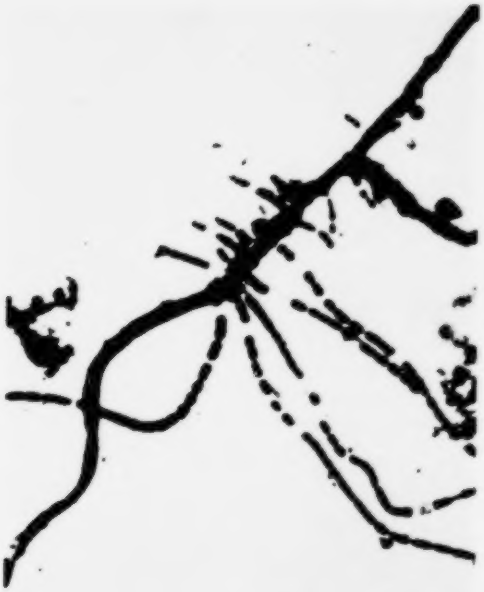


Figure 7 - Crenothrix polyspora. Cells enclosed within a sheath of ferric hydrate and showing false branching. Mag. about 390X. From Harder (16), Plate 3, Fig. B.

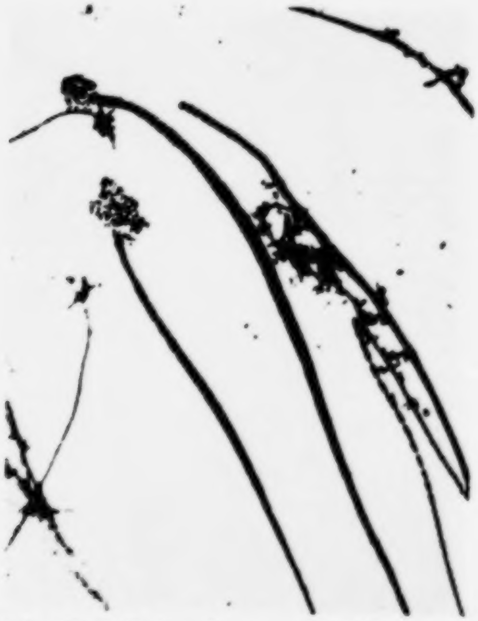


Figure 8 - Crenothrix polyspora. Conidia can be seen inside and coming out at the ends of the filaments. Mag. about 345X. From Cholodny (13), Fig. 5.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

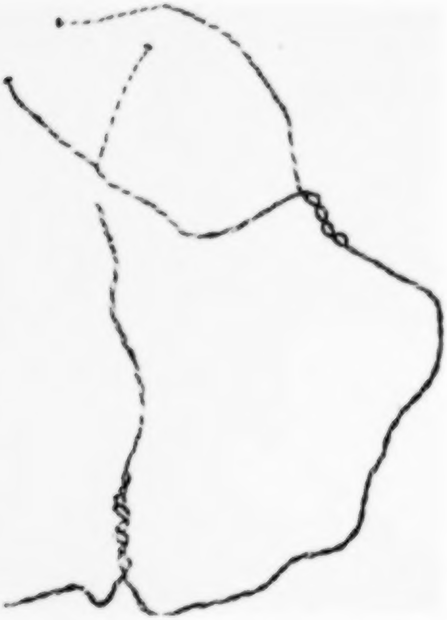


Figure 9 - Gallionella ferruginea. Sketch showing cells at the ends of the long, ribbon-like excretion bands. Mag. about 540X. From Cholodny (13), Fig. 9.

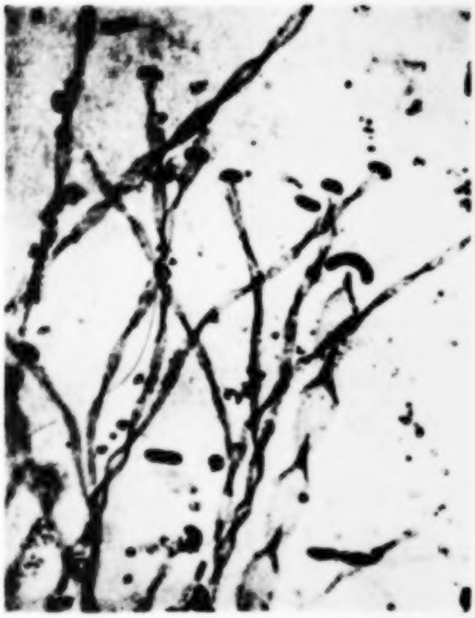


Figure 10 - Gallionella major. Curved cells at the ends of excretion bands. Mag. about 1120X. From Cholodny, Planta, V.8, 1929, Fig. 6.

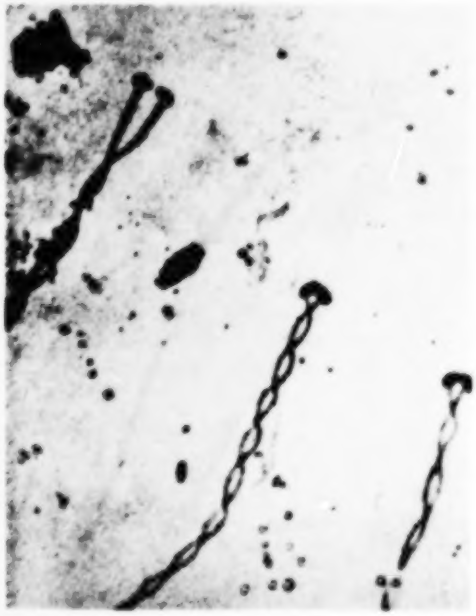


Figure 11 - Gallionella major. Cells at the ends of the excretion bands undergoing division. Mag. about 1180X. From Cholodny, Planta, V.8, 1929, Fig. 3

Figure 12 - Various iron bacteria including Leptothrix and Gallionella in accumulated precipitate of ferric hydrate in gallery water. From Tenny (8), Fig. 4.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

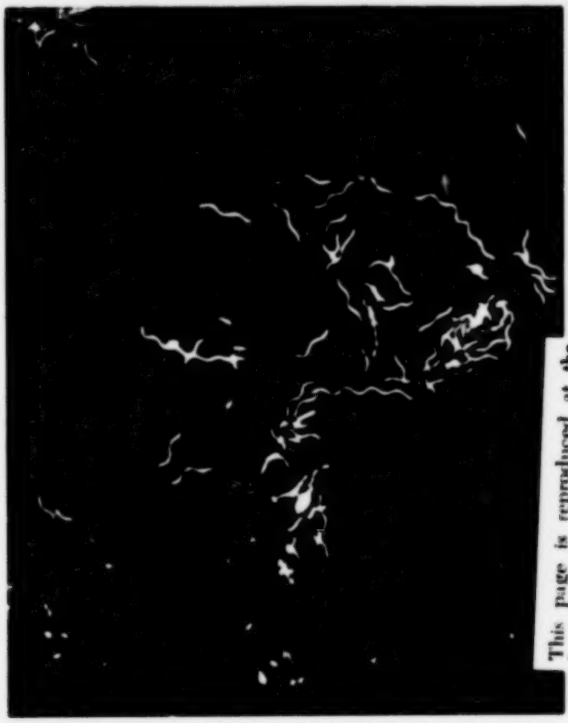


Figure 13 - Spiral cells of the sulfate-reducing bacterium *Sporovibro desulfuricans*. Mag. about 1660X.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

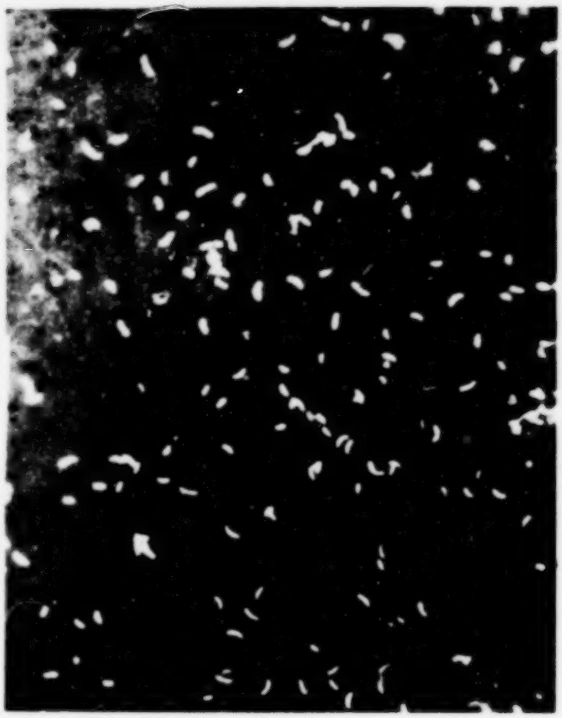


Figure 14 - Curved small cells of *Sporovibrio desulfuricans*. Mag. about 1600X.

Clonothrix. These are filaments attached at the base and showing false branching. The sheath is organic and encrusted with iron. The filaments are broader at the base (5-7 μ) than at the tapering tips (2 μ). The cells are cylindrical and reproduce by spherical conidia which are formed in chains by transverse fission. The type species, *Clonothrix fusca*, has been reported to be a common iron bacterium in water works and pipes carrying water.

Leptothrix (Chlamydothrix). Members of this genus consist of cylindrical cells joined in long filaments enclosed in a common sheath which is first thin and colorless and later thicker, yellow or brown, and encrusted with ferric hydrate. There is very little evidence of a sheath in the young cells, and the sheath of old cells can be almost entirely dissolved with dilute HCl, suggesting that it contains very little organic matter. When the sheath is removed with acid the inner cells are revealed. Reproduction is by cell division, fragmentation of filaments and motile cylindrical swarmer cells measuring 0.7 x 2 to 14 μ. Some species show true branching. Most cells are not attached, whereas some are attached at the base by holdfasts. The filaments of the type species *L. ochracea* have an inner diameter of about 1 μ and a sheath from 0.5 to 1 μ thick. The filaments may attain a length of 1 cm. The brittle and delicate sheath enclosing the cells becomes thickened and brown as iron is deposited. The cells may creep out of old sheaths and leave behind the empty tubes of ferric hydrate. The new cells then form a new sheath. These bacteria are common inhabitants of running and stagnated iron-bearing waters and are encountered in water works. *L. ochracea* is likely to occur in pure well waters and springs which are poor in organic matter, whereas *L. crassa* is found more commonly in slow-flowing water containing some organic matter. Among the species of *Leptothrix* are facultative autotrophs. Some have been cultivated in organic media.

Crenothrix. These are unbranched filaments which are attached at the base. The cells are enclosed in a sheath composed of organic matter which is at first thin and colorless and later becomes thickened and takes on the brown color of the precipitated ferric hydrate. The cells are cylindrical to spherical, dividing in three planes to produce non-motile conidia 1 to 2 μ in diameter. The type species is *C. polyspora*, which differs from other iron bacteria in that it is very large; the filaments may be several millimeters long and 2 μ in diameter. The filaments expand toward the tip where conidia are released. It has been found commonly in wells and water pipes and frequently occurs together with *Gallionella*. It also grows in running water containing organic matter and iron salts and produces thick masses having a brown or green color. It has been reported to be one of the most troublesome iron bacteria in water supplies. *C. polyspora* is probably a facultative autotroph. There has been an unwarranted tendency to label as "*Crenothrix* fouling" any trouble in water supplies caused by filamentous organisms without establishing the nature of the fouling organism.

Gallionella (Spirophyllum). The cells are asymmetrical and secrete ferric hydrate on one side to form a long slender twisted ribbon composed entirely of ferric hydrate. These ribbons serve as stalks which support the bacterial cells. The cells are small, curved, kidney-shaped rods 0.5 x 1.2 µ. Ferric hydrate is secreted from the concave side of the cells. They divide by transverse fission and, after division, each cell produces a secretion filament. At the point where cell division occurs, the secretion strand branches and this gives the erroneous impression that the organism itself is branched. The secretion filaments may attain a length of 200µ or more. The type species is G. ferruginea. Cells of G. major are larger (1 x 3 µ). These bacteria occur in iron-bearing water, are sensitive to organic matter and are believed to be strict autotrophs.

Harder (16) states that Gallionella is one of the most widely distributed iron bacteria, nearly as commonly encountered as Leptothrix. Crenothrix and Clonothrix, he says, are somewhat less common.

Physiology

Although Ehrenberg associated microorganisms with precipitation of iron in 1836 and Cohn presented a theory to explain this precipitation in 1875, particular interest in this phenomenon is traced to later work of Winogradsky whose ideas have dominated the subject as they have that of the physiology of the nitrifying and sulfur bacteria. His first report on the iron bacteria, which appeared in 1888, followed studies of the sulfur bacteria which he had found were able to oxidize sulfur and utilize the energy released by this oxidation for their growth. It is not surprising that he came to the conclusion that iron bacteria were similar physiologically and that they used ferrous iron as a source of energy for development. It was Winogradsky who developed the concept of autotrophic bacteria, with which the iron bacteria and sulfur bacteria are classified. The autotrophic bacteria are characterized by the following physiological properties:

1. They can develop in a medium containing no organic material and they require no externally supplied organic substances.

2. Their carbon requirements can be satisfied by dissolved CO_2.

3. All of the required energy can be obtained from the oxidation of some specific incompletely oxidized inorganic substance.

In the case of strict autotrophic bacteria some organic materials are toxic except at extremely low concentrations and the bacteria are unable to utilize the organic compounds which are generally included in culture media. There are certain facultative autotrophic bacteria which can develop in media containing only inorganic substances but which are able to grow on organic compounds as well.

The reaction by which the iron bacteria are presumed to live as autotrophs is as follows:

$$\begin{aligned} 4\,Fe(HCO_3)_2 + 2\,H_2O + O_2 \\ = 4\,Fe(OH)_3 + 8\,CO_2 \end{aligned}$$

This is an exothermal reaction which is believed to release sufficient energy to satisfy all of the requirements of the bacteria. The strictly autotrophic iron bacteria, such as Gallionella ferruginea, are believed to depend on this reaction, whereas the facultative iron bacteria, including Crenothrix polyspora and some species of Leptothrix, are not limited to growth at the expense of ferrous iron but can also grow on organic compounds. Thus, those bacteria which are able to grow on either ferrous iron or organic materials may produce large amounts of precipitated ferric hydrate when they live on the ferrous iron, and produce accumulations of filamentous fouling material under conditions where they develop on the organic constituents of the water. In the former case they live as autotrophic iron bacteria and in the latter case they grow as heterotrophic bacteria.

In his initial studies on iron bacteria, Winogradsky presented convincing evidence in support of his conclusions that they oxidize ferrous iron in their cells, precipitate the resulting ferric hydrate outside the cells, and utilize the energy liberated by the oxidation reaction. His explanation of the mechanism of precipitation of iron has received additional support from the results of numerous studies, particularly those of Lieske and Cholodny, but it has not been universally accepted, and many theories have been advanced to explain the nutritional peculiarities of the iron bacteria. The results of Molisch (18) and others, the latest being Cataldi (12), have shown that many of the iron bacteria are able to grow on organic materials, and there is reason to believe that at least in the case of certain organisms classified as iron bacteria, precipitation of iron is the result of the decomposition of organic compounds of iron and not the oxidation of inorganic iron compounds.

The reason why progress in the study of the physiology of the iron bacteria has been slow is that it is very difficult to cultivate these organisms in laboratory media. With a few exceptions, they cannot be grown on ordinary media, and there are no known simple procedures for cultivating them. As a result, in most cases, studies have been limited to microscopic observations.

A brief consideration of some of the factors affecting growth of iron bacteria may aid in suggesting the conditions under which they are likely to be encountered, as well as give some indication of their physiological characteristics.

Temperature. Some of the iron bacteria show preference for low temperatures. According to Lieske, G. ferruginea grew under laboratory conditions from 0° to 22° C. with an optimum of 6° C. It has been commonly encountered in springs and cool waters but also in water pipes. Volkova (19) found Gallionella in mineral springs with temperatures as high as 27 to 32 C. but not at 40 to 50 C. Species of Leptothrix have also been found in waters at 1 to 2 C. but their optimum is reported to be higher than that of Gallionella. Lieske and Molisch obtained good growth of Leptothrix at 25 C. Others reported the optimum temperature as 15 C.

Light. The iron bacteria are not sensitive to light and have been found in exposed locations and in shade as well as within pipes where light is completely excluded.

Concentration of iron. The iron bacteria are greatly affected by the presence of dissolved iron but they tolerate considerable differences in iron concentration. Though they have been found in waters with from 10 to 30 ppm iron, they also occur where the amount of iron is as low as from 0.1 to 0.3 ppm., particularly in moving water where the supply of iron is continuously renewed. Harder (16) reports active development of iron bacteria in pipes carrying water with 1.3 ppm. iron. Halvorson (31) found them in springs having 1 to 10 ppm. Schorler (7) records incrustation of 10-cm. pipes carrying water with from 0.2 to 0.3 ppm. iron. In 30 years a deposit was formed 3 cm. thick, leaving an opening only 4 cm. in diameter. In contrast, Knudsen (4) states that to cause tuberculation the water must contain 2 ppm. or more. It has been stated that fouling by iron bacteria occurs only with water containing at least 0.2 ppm. iron. It may be concluded that, though iron bacteria are most likely to appear in water with relatively high iron content, they may develop in water with low iron concentration if the supply of iron is renewed in flowing water.

The general occurrence of iron bacteria in iron-bearing waters is one of the strongest arguments in favor of the idea that the iron plays some role of importance in the development of the bacteria and that the precipitation of iron is the result of some reactions intimately related to their physiology.

Reaction. The influence of reaction on iron bacteria is not definitely established, but there are indications that for many of the organisms slightly acid reactions, such as those caused by solution of considerable amounts of CO_2, are most favorable. Slight acidity increases the iron-carrying capacity of water. With regard to tuberculation, Reddick and Linderman (6) state: "Our investigations to date lead us to believe that an alkaline iron-bearing water, having only a small content of organic matter, will not tuberculate, and that a slightly acid water containing iron and having a high organic matter content will readily tuberculate." Knudsen (4) also concluded that tuberculation was more likely to occur in slightly acid waters. On the other hand, Volkova (19) found Gallionella in mineral springs having a pH of from 7.0 to 7.6. Wilson (9) reports a case of profuse development of Crenothrix in a large main which carried water nearly free of iron and manganese and having a pH of from 7.3 to 8.0. The organism grew in gelatinous masses, and it was presumed that the iron was derived from the pipe through the bases of the bacteria. In this case there is reason to suspect that the water contained organic materials which supported growth of the bacteria. Hardman and Henricci (17) recovered Siderocapsa regularly from alkaline hard water lakes but never from neutral or acid soft water lakes. These authors, as well as Cholodny, express the opinion that iron precipitates only incidentally during the development of these bacteria and that organic matter supplies their energy requirements for growth. Cataldi (12) cultivated Sphaerotilus and Leptothrix at pH 6.8 but organic media were used and conditions were not representative of those under which iron becomes precipitated. Wilson (10) controlled fouling by Crenothrix by making the water alkaline (pH 8.5) with sodium carbonate. Control may have been effected by making the reaction unfavorable to the iron bacteria, or it may have been due to the elimination of iron, manganese or organic matter upon which the bacteria depended for growth.

Oxygen. The iron bacteria are aerobic and as such are dependent on the presence of free oxygen. In stagnant water they are found only at the surface but they may find conditions favorable for growth below the surface adjacent to green plants which liberate oxygen through photosynthesis.

Organic matter. There is much conflicting evidence regarding the influence of organic materials on iron bacteria, but it has been definitely established that some of these organisms can grow on organic compounds, and it appears probable that at least some of the organisms which are now classified with iron bacteria actually require organic matter for growth. Sphaerotilus (Cladothrix) has been repeatedly grown on organic media, first in 1904 by Adler who used iron ammonium citrate, and subsequently by Molisch on mangan peptone, iron ammonium tartrate and other organic compounds. Most recently, Cataldi (12) reported that Sphaerotilus, as well as Leptothrix, was readily cultivated on organic media, but that it failed to grow in mineral media in which ferrous and manganous salts were used as the only sources of energy. Molisch carried L. crassa through 47 successive transfers in a nutrient solution containing from 1 to 2 per cent peptone to which no iron was added. Under these conditions the cells were colorless and had no iron in their delicate sheaths.

Although these results indicate that some of these bacteria can grow in the absence of organic matter, they do not prove that the bacteria are unable to grow by the oxidation of ferrous iron under suitable cultural conditions. In fact, it is significant that no one has succeeded in obtaining growth of Gallionella in organic media. Furthermore, Lieske found that certain organic compounds were actually toxic. Concentrations of from 0.25 to 0.35 per cent of asparagin, sucrose and peptone inhibited growth; some depression was noted even at 0.01 per cent. This response is similar to that of some other autotrophic bacteria and suggests that *Gallionella* may indeed be strictly autotrophic. *Gallionella* has been encountered, nevertheless, in waters containing considerable organic matter, such as water in aquariums and ditches, as well as in springs and streams which are relatively free of organic matter.

On the basis of the scattered evidence of the influence of organic matter on the iron bacteria it may be tentatively concluded that *Sphaerotilus*, *Siderocapsa* and *L. crassa* require organic matter and may be strictly heterotrophic, that *Crenothrix* and *Leptothrix ochracea* are facultative autotrophs and that *Gallionella* is strictly autotrophic. In waters containing relatively large amounts of organic matter it is common to encounter *Crenothrix* and *Leptothrix*, whereas in water low in organic matter *Gallionella* is prominent.

Sources of nitrogen. The amounts of nitrogen required by the iron bacteria are very small compared to the requirements for iron and it is probable that the nitrogen content of most waters meets their needs. There is no specific information regarding the nitrogen compounds that they use, but some information can be obtained by checking the constituents of the culture media which have been used to grow the bacteria. Some of the media contained complex and diverse organic compounds of nitrogen, such as beef extract, peptone and plant extracts. Iron bacteria have also been cultivated in simpler media containing iron ammonium citrate and similar compounds and presumably the bacteria used the ammonium nitrogen to meet their requirements. Lieske stated that he succeeded in growing a culture of *Leptothrix* in a strictly mineral medium in which the nitrogen was supplied as ammonium sulfate. The medium in which he grew *Gallionella* contained both ammonium and nitrate nitrogen and it cannot be stated which was used by the organism. No definite conclusion can be drawn from such incomplete information except that certain bacteria can use inorganic nitrogen. It is of interest to note, however, that with some of the autotrophic bacteria ammonium nitrogen is preferred or required and that nitrate nitrogen and certain amino acids are even toxic. With some other bacteria as well, such as the methane bacteria, ammonium nitrogen is used, whereas nitrate is toxic.

Transformation of Iron by Nonspecific Bacteria

In most discussions of iron precipitation by microorganisms, principal attention has been devoted to the iron bacteria, in fact almost to the exclusion of all other microbial activities which may be directly or indirectly related to the phenomenon. This is an oversimplification of the subject and inadequately evaluates other important bacterial activities. As with all transformations in nature, iron precipitation in water occurs in the presence of numerous microorganisms and under conditions where various strictly chemical reactions may alter the solubility of iron.

In cases where the transformations of iron are due to non-specific bacteria, the iron has no particular significance in the metabolism of the bacteria but is altered as a result of changes in the medium brought about by bacterial development. From the following reactions it is possible to make some predictions regarding the changes which may occur as a result of changes in reaction (pH) as well as those controlled by oxidation and reduction (33):

In the presence of solid ferric hydrate

$$\frac{[Fe^{++}]}{[H^+]^2} = \frac{K}{[O_2]^{0.25}}$$

Under atmospheric conditions or with constant oxygen pressure the reaction becomes

$$\frac{[Fe^{++}]}{[H^+]^2} = K'$$

In cases where the solution is saturated with iron, where there is an excess of solid ferric hydrate and where the oxidations by molecular oxygen are reversible, changes in the hydrogen-ion concentration and the oxygen pressure (degree of oxidation or reduction) will result in changes in the amounts of iron in solution. With a decrease in the oxidizing intensity, but at constant pH, there will be an increase in the concentration of ferrous ions. Since ferrous iron is more soluble than ferric iron, some of the solid ferric hydrate will go into solution and become reduced. With increasing oxygen pressure at constant hydrogen-ion concentration there will be a tendency for ferrous iron to become oxidized to ferric and, if the solution was initially in equilibrium, the increase in ferric iron will result in precipitation and a decrease in the amount of iron in solution.

With increase in hydrogen-ion concentration, but at constant oxygen pressure, there will also be an increase in the amount of dissolved ferrous iron through reduction of some of the ferric iron, which will cause some of the solid ferric hydrate to go into solution. With decrease in the hydrogen-ion concentration, there will be a tendency for ferrous iron to be oxidized to ferric iron and precipitate. Some of the data in Table 1 show that during bacterial development in organic media, where conditions became strongly reducing because of lack of diffusion of oxygen, iron went into solution. In this experiment culture media treated with either dextrose or peptone, with or without ferric hydrate, were inoculated with soil and incubated for three weeks. The medium nearly filled the culture flasks and thus suppressed diffusion of oxygen. As shown in the next to the last column, considerable iron was dissolved in the dextrose medium. This was due in part to the production of acid and it can be noted that the iron exerted a buffering effect and prevented the shift to the low pH shown in the medium containing no iron. The reaction of the peptone medium remained nearly constant irrespective of the presence of ferric hydrate and, even though the pH was close to neutrality,

TABLE 1

Influence of Bacterial Development on Solution and Precipitation of Iron

Medium	pH		Fe^{++} in Solution, *mg./100 ml.*	
	Anaerobic	Aerobic	Anaerobic	Aerobic
Dextrose	3.0	3.1 3.1*	0	0 0*
Dextrose + $Fe(OH)_3$	6.0	5.3 4.2*	71.88	12.50 11.88*
Dextrose + $Fe(OH)_3$	6.0	4.6 4.3*	73.75	21.88 12.50*
Peptone	6.2	8.0 7.8*	0	0 0*
Peptoe + $Fe(OH)_3$	6.4	8.2 8.0*	22.98	0 0*
Peptone + $Fe(OH)_3$	6.4	7.7 7.7*	16.25	0 0*

* Cultures treated with phenol at time of exposure to free air.

appreciable iron remained in solution. The results show that iron was dissolved under anaerobic conditions not only during decomposition of the carbohydrate where there was an increase in the acidity, but also during decomposition of nitrogenous materials where there was relatively little change in pH.

After the cultures had developed for three weeks, the solutions were poured into flat vessels to provide large surfaces in contact with the air. During the two-week period that these cultures were kept under aerobic conditions all of the iron precipitated from the peptone solutions and a large part disappeared from the dextrose media, but some iron still remained in solution. These changes were independent of the action of bacteria, since phenol-treated solutions behaved the same as those in which no attempt was made to control bacterial development. Precipitation was doubtless due to an increase in the oxidizing intensity. It is of interest that there was an increase in the hydrogen-ion concentration in both the sterile and non-sterile dextrose solutions during aerobic treatment.

Even though the solutions which initially contained dextrose became more acid during exposure to the air, less iron was held in solution. This may seem contradictory, since an increase in the hydrogen-ion concentration favors solution of iron. The changes in amounts of dissolved iron were caused by an increase in the oxidizing intensity and not the hydrogen-ion concentration. In fact, the completeness of precipitation of the iron during exposure to the air was probably limited somewhat by the acidity; if the reaction had remained close to neutrality, it is probable that all of the iron would have been precipitated.

Changes such as these are not due to the action of any specific bacteria nor are they caused by bacteria alone, but they can be effected by various microorganisms and the extent of the changes is proportional to the alteration in the reaction and in the degree of oxidation. The principal effect of the bacteria in the experiment mentioned above was the solution of iron; the precipitation was the result of strictly chemical reactions. It can be concluded that generally an increase in the oxygen pressure or a decrease in the hydrogen-ion concentration leads to oxidation of iron and its precipitation.

Precipitation of iron by algae and other water plants may be cited as another example of a reaction which has no direct connection with the growth of the organism responsible for the change. These green plants liberate oxygen by photosynthesis and thus provide more strongly oxidizing conditions in the water, leading to the oxidation and precipitation of iron on the plant surfaces This type of reaction is relatively common in iron-bearing waters.

Mention should be made also of some of the changes which are caused by differences in the concentration of dissolved carbon dioxide (31). This gas is produced in such abundance that in some subterranean waters it greatly affects the solubility of iron. It is also produced in quanities by microorganisms, by animals and by higher plants in the dark. Relatively large amounts of CO_2 may be removed from solution by green plants during photosynthesis. The mixed population of microorganisms in general tends to increase the carbon dioxide concentration.

In order to follow the influences of carbon dioxide on iron concentrations, consider a solution which is saturated with CO_2 and in which there is an excess of solid ferrous carbonate. If CO_2 is removed from such a solution some of the solid ferrous carbonate goes into solution to replace the dissolved CO_2 . This increases the ferrous iron concentration above the stable value and results in its oxidation to ferric iron followed by its precipitation as ferric hydrate. As more CO_2 is removed this sequence of events continues until all of the solid ferrous carbonate has become dissolved. The amount of iron remaining in solution at the time when the CO_2 has all been removed would be determined principally by the reaction (pH) of the solution. The more acid the solution the greater would be the amount of dissolved iron. One would expect that there would be a slight decrease in the hydrogen-ion concentration as the CO2 was removed after the last portion of solid ferrous carbonate had gone into solution.

If CO_2 were added to a solution which was saturated with ferric hydrate and in which there was an excess of ferric hydrate, there would be at first a slight increase in acidity. Some ferric hydrate would go into solution and a portion of this would be reduced to ferrous carbonate. Thus, in water stored under conditions where there is an increase in the CO_2 concentration, conditions are favorable for solution of iron. As more ferrous carbonate was produced with the addition of more CO_2, the solution would become saturated, and further addition of CO_2 would result in the precipitation of ferrous carbonate. Continuous addition of CO_2 would cause solution of more ferric hydrate and result in its reduction and subsequent precipitation as ferrous carbonate. This would continue until all of the ferric hydrate had become dissolved.

Thus by decreasing the partial pressure of CO_2 in the atmosphere below that of the solution there will be a tendency for CO_2 to come from the solution and this will favor precipitation of ferric hydrate. In addition, when the oxidation-reduction potential is increased there will be a tendency for oxidation and precipitation of iron. Consequently, when subterranean waters come to the surface, conditions are favorable for both of these changes. In many cases this is responsible for the precipitation of ferric hydrate from the iron-laden waters issuing from the earth. The CO_2 escapes and there is some decrease in acidity, and this is followed by oxidation of ferrous to ferric iron which is precipitated as ferric hydrate. These changes can occur in the absence of microorganisms but it is possible that various organisms accelerate the reactions.

In studying the iron bacteria and other microorganisms concerned in iron precipitation, organic media have been frequently used and, as mentioned earlier, ferric ammonium citrate is one of the common medium constituents. Although large amounts of organic compounds of iron may remain in solution at alkaline reactions, they remain soluble by reason of the fact that they are poorly ionized. If such compounds are decomposed by microorganisms, ionic iron is released in the medium and precipitates as ferric hydrate. There is nothing specific about the organisms concerned in this transformation except their ability to decompose the organic portion of the compound. Studies with ferric ammonium citrate are particularly informative in this connection. *Escherichia coli* and *Aerobacter aerogenes* differ in ability to decompose citrate. If flasks of a culture medium containing ferric ammonium citrate are inoculated with these two bacteria, iron will precipitate from the solution inoculated with *Aer. aerogenes*, but not from the one inoculated with *Esch. coli*. Iron precipitation by phenomena of this type is widespread. Many different organic compounds of iron occur in natural waters and various bacteria are concerned in the precipitation of iron from them.

In certain cases, the opposite may occur. During decomposition of organic matter by microorganisms, iron may combine with some of the products of decomposition and form stable organic compounds. Thus, in certain locations iron may go into solution through microbial action and be precipitated some distance away through breakdown of the initially produced organic compounds.

Consideration of the various ways in which microorganisms can affect the solution and precipitation of iron leads to the conclusion that iron bacteria are not the only agents of iron transformation but that various other bacteria may be of considerable importance.

Sulfur Bacteria

Some of the sulfur bacteria are occasionally encountered in water, particularly in water containing sulfide or elemental sulfur. In exceptional cases filamentous sulfur bacteria have been troublesome as fouling organisms. Other sulfur bacteria may cause corrosion.

The sulfur bacteria as a group are characterized by their ability to oxidize sulfur or various sulfur compounds, generally to sulfate, and to utilize these reactions in their development. There are morphologically different sulfur bacteria, varying from the complex filamentous organisms, some of which are pigmented and photosynthetic (20, 24), to the small simple bacteria which are hardly distinguishable morphologically from *Esch. coli* (23). The filamentous forms are typical water organisms and are sometimes encountered together with iron bacteria in water basins and even in water distribution systems. Little need be said regarding these organisms except that they may contribute to the formation of slimes and possibly to the tastes which develop in water where there is growth and breakdown of the slime films.

One of the typical, simple sulfur bacteria may bring large amounts of iron into solution under conditions favorable for its development. This is *Thiobacillus thiooxidans*, an aerobic bacterium which has the capacity of oxidizing sulfur and certain incompletely oxidized sulfur compounds with the production of very high acidity (22). It is able not only to tolerate high acidity but to produce the acid as well. It has been recovered principally from soils and sulfur springs but it may also be encountered in water under special conditions. In several instances it was isolated from soils where the acidity was so extreme as to indicate an abnormality. In artificial media it has been cultivated at reactions close to pH 0 and it has produced acidity as high as 10 per cent sulfuric acid.

One of the most remarkable phenomena in which this organism played an important part was that recently reported by Harmsen (21), dealing with changes in some soils of the Wieringen Polder, an area of land reclaimed from the Zuiderzee in Holland. The bottom material of this arm of the ocean was black with sulfide. When the area was diked, the water pumped out and the soil drained, the sulfide underwent change, partly chemical and partly bacteriological. Some of the sulfide was oxidized to elemental sulfur, which was then attacked by *T. thiooxidans*. In locations where there was an abundance of lime in the soil the sulfuric acid which was formed became neutralized, as a relult of which the rate of reclamation was greatly accelerated. In locations where there was little or no lime there was a rapid increase in acidity and large amounts of iron were dissolved from the soil materials. This went on to such an extend that quantities of basic ferric sulfate accumulated in the soils and gave them a characteristic yellowish to gray color. In some of the poorly buffered sandy soils the reaction became exceedingly acid and in certain cases even had a negative pH.

It has been reported that these bacteria are concerned in the corrosion of iron pipelines. One case has been reported where a calking compound which contained sulfur was responsible for the trouble. When the sulfur was attacked by *T. thiooxidans* sufficient acid was produced to corrode the pipe severely at the joint.

Abnormally high acidity (below pH 2.0) has in the past served as a clue leading to the detection of this bacterium and to its identification as the agent responsible for the acid condition.

Sulfate-Reducing Bacteria

The sulfate-reducing bacteria are responsible for still other iron transformations than those discussed thus far. Sulfide, which is one of the principal products of their development, reacts with dissolved iron or metallic iron to form black insoluble iron sulfide. Furthermore, the bacteria have assumed particular significance since is was discovered that they are agents of corrosion.

They are not the only bacteria which form sulfide but they produce most of the sulfide of bacterial origin. Some sulfide is produced by various bacteria during decomposition of organic sulfur compounds under conditions of deficient aeration such as occur in sewage. Sulfide can also be formed by numerous bacteria by reduction of sulfite, thiosulfate, elemental sulfur and other incompletely oxidized inorganic sulfur compounds. Most of the sulfide in black muds of ocean sediments and tidal basins, as well as in poorly drained heavy soils, comes from the reduction of sulfate.

The bacteria which reduce sulfate to sulfide are of considerable interest, particularly because of their ability to effect this reaction. So far as is known there is only one specific group of bacteria which can reduce sulfate to sulfide. Thus, the sulfate-reducing bacteria are a highly specialized physiological group. Furthermore, they are morphologically distinct. They are curved cells, occasionally having a spiral shape. They are actively motile and form spores under suitable conditions. From time to time they have been listed under the genera *Spirillum*, *Microspira*, *Vibrio*, *Desulfovibrio* and, most recently, *Sporovibrio*. The most common species are *Sporovibrio desulfuricans* and *S. rubentschickii*. They are strict anaerobes and are heterotrophic. Sulfate is the specific hydrogen acceptor which serves the same purpose as free oxygen in the development of strict aerobic bacteria. Organic materials which provide energy for the growth of the sulfate-reducing bacteria become oxidized by the sulfate which is reduced to sulfide. One of the typical reactions whereby lactate is broken down to acetate and CO_2, and whereby sulfate is reduced to sulfide, is shown below:

$$\begin{aligned} &\underset{\text{Sodium lactate}}{2\,CH_3.CHOH.COONa} + CaSO_4 \\ &\quad = \underset{\text{Sodium acetate}}{2\,CH_3.COONa} + H_2S \\ &\qquad + CaCO_3 + CO_2 + H_2O \end{aligned}$$

This reaction is exothermal and can provide all the energy necessary for development of the bacteria. Many different compounds can be used, including numerous organic acids, carbohydrates, alcohols, amino acids and more complex organic nitrogenous substances.

The different species of sulfate-reducing bacteria are alike morphologically as far as is known and they differ only in the organic compounds which they are able to attack (28).

These bacteria are of some importance in water distribution systems in that they produce sulfide which is dissolved in the water and makes the water objectionable by reason of the odor, the presence of suspended black particles and the corrosive effect of the sulfide on steel and other metals. They are of still greater importance as corrosive agents through another mechanism of action.

Their relationship to this corrosion process was discovered in 1934 by von Wolzogen Kühr (30) who showed that these bacteria cause corrosion under anaerobic conditions where it would be expected that the iron would remain passive. He was led to conclude that the bacteria were responsible for corrosion, since iron sulfide was one of the principal products of corrosion; that sulfate was reduced during the process; that hydrogen was taken up by microorganisms where corrosion occurred; and that the sulfate-reducing bacteria were active in the anaerobic soils and environments where corrosion occurred. There is nothing unusual in the bacterial corrosion process that distinguishes it from the generally accepted electrochemical mechanism of corrosion except that the bacteria serve to activate the cathodic hydrogen. Under aerobic conditions this hydrogen is oxidized by oxygen of air in the process of rusting, as shown below.

Under conditions where oxygen is excluded, the system becomes polarized, since the hydrogen which accumulates at the cathode is not removed. In the absence of some depolarizing agent there is no corrosion. The sulfate-reducing bacteria serve as depolarizing agents under anaerobic conditions by removing the hydrogen and oxidizing it at the expense of sulfate which is reduced to sulfide. The reactions on page 980 were proposed to explain this transformation.

As will be seen from the last equation, the products of corrosion are ferrous sulfide and ferrous hydrate, the quantities of which are in the proportion of 1 to 3. Following von Wolzogen Kühr's study of the process, others have sought to establish whether or not anaerobic corrosion was a factor of importance in various locations throughout the world (25, 26, 29). Principal interest has been focused on corrosion in soil. Results of numerous studies have indicated that this type of corrosion is not only widespread but may be so severe as to cause pipe failure within a period of a few years. A steel pipe 200 mils thick may be penetrated in eight to ten years under conditions where this corrosion is severe (26).

With steel the metal is pitted and frequently the pits are sufficiently close together to coalesce and cause slab corrosion. Under this condition a large portion of the pipe surface may lose metal, but at different rates so that the metal surface is very uneven. When a corroding steel surface is exposed, the spots where corrosion had occurred appear as raised areas where the corrosion products had accumulated.

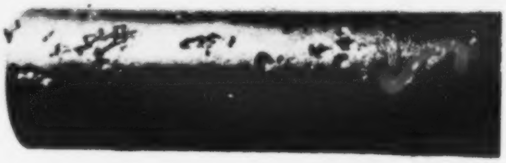


Figure 15. 6-in. steel pipe pitted by anaerobic corrosion during eight years burial in soil - Specimen provided by R.F. Hadley.

There is frequently a hard black crust of corrosion products, beneath which is softer material which is black or mixed black FeS and white ferrous hydrate. When the corrosion products are removed the metal surface is bright and silvery. There is no tendency for the corrosion products to adhere tightly to the metal surface and thus to stifle corrosion, as sometimes occurs with rusting.

With cast iron a condition is encountered which has been called "graphitization." Iron showing such attack is somewhat soft and can be penetrated with a knife. This is caused as a result of loss of iron from the pipe, leaving a porous residual structure consisting of a mixture of iron and carbon which resembles graphite. When the pipe dries out this material hardens. Graphitization does not always characterize anaerobic corrosion of cast iron. In some cases there is a heavy accumulation of corrosion products which may be an inch or more in thickness and contain an abundance of ferrous sulfide. Beneath this crust the metal is bright but not noticeably softened. In such cases it seems likely that most of the iron has come from the surface of the pipe.

This type of corrosion is not confined to soils, although most of the studies have been made on metal buried in soils. It also occurs in fresh water and salt water, as well as in the sediments and bottom materials of lakes, rivers and oceans. It has been encountered in various tanks where sediment covered the steel surface or where the metal was incompletely coated so as to exclude oxygen or oxygenated water

$$\left.\begin{aligned} 4HOH &\rightleftharpoons 4H^{\cdot} + 4OH' \\ 2Fe + 4H^{\cdot} &\rightleftharpoons 2Fe^{\cdot\cdot} + 4H \end{aligned}\right\}\text{Anodic solution of iron}$$

$$4H + O_2 \rightarrow 2HOH \qquad \text{Depolarization}$$

$$\left.\begin{aligned} 2Fe^{\cdot\cdot} + 4OH' &\rightarrow 2Fe(OH)_2 \\ 2Fe(OH)_2 + \tfrac{1}{2}O_2 + H_2O &\rightarrow 2Fe(OH)_3 \end{aligned}\right\}\text{Corrosion products}$$

$$3HOH + 2Fe + 1\tfrac{1}{2}O_2 \rightarrow 2Fe(OH)_3 \qquad \text{Summary}$$

without preventing exchange of salts and organic materials contained in the water. Anaerobic corrosion is important in sewerage equipment where anaerobic conditions prevail.

Conditions are more readily controlled in domestic water distribution systems than in sewage where there is intense bacterial activity supported by the abundance of organic matter. Pomeroy (27) reported an interesting case where an 8-in. cast-iron pipe became nearly filled with corrosion products consisting principally of iron sulfide and iron oxide. After 13 years only a 3-in. opening remained. The conditions under which the corrosion occurred and the nature of the corrosion products suggest that there had been anaerobic corrosion.

$$\begin{array}{rl}
\left.\begin{array}{r} 8\mathrm{HOH} \rightleftharpoons 8\mathrm{H}^{\cdot} + 8\mathrm{OH}' \\ 4\mathrm{Fe} + 8\mathrm{H}^{\cdot} \rightleftharpoons 4\mathrm{Fe}^{\cdot\cdot} + 8\mathrm{H} \end{array}\right\} & \text{Anodic solution of iron} \\
\mathrm{CaSO_4} + 8\mathrm{H} \rightarrow \mathrm{H_2S} + 2\mathrm{HOH} + \mathrm{Ca(OH)_2} & \text{Depolarization} \\
\left.\begin{array}{r} \mathrm{Fe}^{\cdot\cdot} + \mathrm{H_2S} \rightarrow \mathrm{FeS} + 2\mathrm{H}^{\cdot} \\ 3\mathrm{Fe}^{\cdot\cdot} + 6\mathrm{OH}' \rightarrow 3\mathrm{Fe(OH)_2} \end{array}\right\} & \text{Corrosion products} \\
\hline
4\mathrm{Fe} + \mathrm{CaSO_4} + 4\mathrm{HOH} \rightarrow \mathrm{FeS} + 3\mathrm{Fe(OH)_2} + \mathrm{Ca(OH)_2} & \text{Summary}
\end{array}$$

There is still another type of corrosion associated with development of both sulfate-reducing bacteria and sulfur bacteria which has been encountered in both iron pipes and concrete conduits. In cases where the water contains sulfide or where sulfide is produced as in sewage, and the pipe is only partly filled so that there is an air blanket over the water, some of the sulfide becomes dissolved in the moisture film on the upper walls of the pipe. Here it undergoes oxidation, caused principally by sulfur bacteria, and the sulfuric acid which is formed attacks the pipe, causing its disintegration.

It has been definitely established that under certain soil conditions the exterior of pipes may be attacked by anaerobic corrosion, but little consideration has as yet been given to the possibility that this corrosion is a factor on the inside surface of water pipes. Water is generally well supplied with oxygen and it might seem unlikely that conditions would be favorable for development of anaerobic bacteria. Nevertheless, there are certain conditions under which this type of corrosion may occur, one of which is a metal surface coated with ferric hydrate and other fouling material such as that produced by iron bacteria. Although the organisms which produce the incrustation and accumulate the fouling material are not themselves corrosive, there is some evidence that corrosion occurs beneath the nodular accumulations. This corrosion may be the result of electrolysis caused by differential aeration, but it seems more likely that in some cases at least there has been anaerobic corrosion. The mixture of ferric hydrate and organic materials could serve as a barrier preventing oxygenated water from reaching the metal surface and thus provide anaerobic conditions. The accumulations should not be so tight as to exclude contact of the films of water with the metal. Furthermore, there should be an exchange of dissolved materials between the main body of water and the water in contact with the metal. This set of conditions should favor development of anaerobic sulfate-reducing bacteria; they could obtain a supply of nutrient elements from the water films, possibly some organic matter from the same source, and some organic nutrients from residues of microbial cells contained in the layer of material coating the metal.

The possibility of anaerobic corrosion under these conditions is not entirely theoretical. The appearance of the underlying metal in badly fouled pipes suggests that anaerobic bacteria develop at the metal surface. Larson (5) reported that at pipe joints and extending from these points for various distances there was an accumulation of material consisting of a surface crust of hard, yellow-brown material beneath which was black loose slime containing sulfide. He stated, "The outer coating does not appear to be sufficiently impermeable to prevent corrosion, but appreciable inhibition is afforded." The descriptions by Reddick and Linderman (6) and Knudsen (4) of the tubercles on water mains leave little doubt regarding the presence of sulfate-reducing bacteria in the tubercles. Furthermore, these incrustations were also found to produce conditions favorable for corrosion. The cone-like structures were described as consisting of an outer coating of red ferric oxide mixed with black magnetic iron oxide and of a center which was black and soft and frequently contained sulfide. When the black material was exposed to the air it became oxidized to red ferric oxide. Beneath the tubercles which tended to coalesce and cover large areas there were pitting and other evidences of corrosion.

Methods of Study

The relationships of microorganisms to iron transformations are such that no single set of methods can be used to determine the nature of the organisms or their role in the transformations. The organisms are diverse, and special procedures are required for each group. No routine chemical or cultural tests have been developed such as those which are so effectively used to test water for sewage pollution. In order to determine whether such bacteria as those under discussion are responsible for undesirable conditions in the water or the distribution system it is of primary importance to know the characteristics of these bacteria.

Study of the iron bacteria is complicated by the fact that there are no simple methods for their cultivation. Whereas some of these bacteria have been grown in pure culture in artificial media, this is not readily accomplished. In only a few instances have iron bacteria been grown at all in inorganic media. Their study is consequently limited almost entirely to microscopic observations and simple chemical tests.

Material suitable for examination may be obtained from fouled surfaces, but only fresh material should be used, since the cells become distorted by desiccation and the material in which they are imbedded cannot be readily redispersed. With water which supports active development of iron bacteria, culture material may be obtained by running a slow stream into a glass cylinder or over a trough for several days. A slimy growth may appear in which there are iron bacteria together with precipitated ferric hydrate. This material can then be used for microscopic studies. Culture slides or cover slips can be kept submerged in the running water for days or weeks and the iron bacteria which become attached to the surfaces can be studied. Wilson (9) suggested that glass slides be inserted in pipes in specially devised stuffing boxes in such a manner that the bacteria would be able to develop under conditions which are typical for the inside of the pipes. Periodically the slides could be removed and studied to determine the abundance and type of organisms which become attached to the slides. Metal slides could also be similarly used in cases where it was desirable to make tests under conditions more representative of the pipes themselves.

Microscopic preparations should be first examined in a fresh condition and before treatment with any chemical. The material could be subsequently treated in various ways to bring out the characteristics of the bacterial cells. Gallionella can be stained with crystal-violet or carbol-fuchsin in a wet preparation in order to reveal the terminal curved cells at the ends of the bands of ferric hydrate excreted by the bacteria. Many of the iron bacteria are so coated with deposits of ferric hydrate that the characteristics of the cells cannot be distinguished until the preparations are treated with dilute acid which removes most of the ferric hydrate. Acid treatment is not desirable in the case of Gallionella since this dissolves the characteristic bands of excreted material. Preparations of other iron bacteria which have been treated with acid and subsequently washed in water can be stained with the common bacteriological stains or with dilute iodine solution which stains the cells very lightly and reveals cell structures which are obscured when other stains are used.

The presence of iron in the accumulated material where the bacteria develop is generally so obvious that it is not necessary to test for it but it may be desirable to test for the relative abundance of iron and manganese in the products.

Where attempts are made to grow the organisms it should be kept in mind that the iron bacteria are aerobic, and that many of them grow best at neutral or slightly acid reactions, and that the temperature of incubation should be fairly low. Some of the bacteria grow best at 10° C. or less. The methods which have been most successfully used to cultivate the iron bacteria have been summarized by Cholodny (14).

Study of the sulfur bacteria also requires the use of special methods. Some of the higher filamentous sulfur bacteria can be recognized by the occurrence of highly refractive globules of sulfur within the cells (20, 24). They may appear in sulfide-containing water which is brought in contact with the air. During growth of these bacteria there is a decrease in the sulfide, an increase in the acidity and an increase in the amount of sulfate, all of which can be tested. Though the filamentous sulfur bacteria are not readily cultivated in artificial media, some of the simple sulfur bacteria will grow in special solution media (22,23) and their relative abundance can be determined.

The anaerobic sulfate-reducing bacteria are also a distinct physiological group of organisms. Their development is characterized by the accumulation of sulfide which reacts with iron to produce black iron sulfide. Water in which they develop may become black or have a sulfide odor. In special cases the activity of these organisms is confined to certain areas of the pipe surface. At these locations there is black material which should give a positive test for sulfide. Sulfate-reducing bacteria can also be cultivated in special media (28), by use of which their relative abundance can be determined and their morphological characteristics ascertained.

REFERENCES

Iron Bacteria As Fouling Organisms

1. Alexander, L. J. Control of Iron and Sulfur Organisms by Super-chlorination and De-chlorination. Jour. A.W.W.A., 32: 1137 (1940) ; 36: 1349 (1944).

2. Brown, K. W. The Occurrence and Control of Iron Bacteria in Water Supplies. Jour. A.W.W.A., 26: 1684 (1934).

3. Carroll, E. B. Control of Iron Bacteria by Super-chlorination. Jour. A.W.W.A., 33: 168 (1941).

4. Knudsen, H. A. Corrosion and Tuberculation. Jour. A.W.W.A., 32: 387 (1940).

5. Larson, T. E. Bacteria, Corrosion and Red Water. Jour. A.W.W.A., 31: 1186 (1939).

6. Reddick, H. G. And Linderman, S. E. Tuberculation of Mains as Affected by Bacteria. J.N.E.W.W.A., 46: 146 (1932).

7. Schorler, B. Die Rostbildung in den Wasserleitungsröhren. Centr. Bakt. II (Ger.), 15: 564 (1906).

8. Tenny, M. K. Des Moines Iron Problem. Jour. A.W.W.A., 31: 96 (1939).

9. Wilson, C. Bacteriology of Water Pipes. Jour. A.W.W.A., 37: 52 (1945).

10. __________. Crenothrix: Alive, Dead and Entombed. W. W. & Sew., 92: 90 (1945).

11. Beger, H. Leptothrix echinata, ein neues vorwiegend Mangan fällendes Eisenbakterium. Zentr. Bakt. II (Ger.), 92: 401 (1935).

12. Cataldi, M. S. Estudio Fisiologico y Sistematico de Algunas Chlamydobacteriales. Thesis, Buenos Aires, Argentina, 96 pp. (1939).

13. Cholodny, N. Die Eisenbakterien. (Ger.) Gustav Fischer, Jena. 162 pp. (1926).

14. _______. Methoden zur Kultur der Eisenbakterien. Handbuch d. Biol. Arbeitsmethoden (Ger.) (E. Abderhalden) 12: 2: 889 (1935).

15. Dorff, P. Die Eisenorganismen. (Ger.) Gustav Fischer, Jena. 62 pp. (1934).

16. Harder, E. C. Iron-Depositing Bacteria and Their Geologic Relations. U:S. Geol. Survey, Prof. Paper 113, 89 pp. (1919).

17. Hardman, Y. And Henrici, A. T. Studies of Fresh-Water Bacteria. V. The Distribution of Siderocapsa treubii in Some Lakes and Streams. J. Bact., 37: 97 (1939).

18. Molisch, H. Die Eisenbakterien. (Ger.) Gustav Fischer, Jena. 83 pp. (1910).

19. Volkova, O. Y. Ferrobacteria of Caucasian Mineral Springs and Their Role in the Formation of Ferrous Deposits. Microbiology (U.S.S.R.) 8: 863 (1939).

Sulfur Bacteria

20. Bavendamm, W. Die farblosen und roten Schwefelbakterien. (Ger.) Gustav Fischer, Jena. 156 pp. (1924).

21. Harmsen, G. W. Biologische ijzeromzettingen in den boden. (Neth.) Chem. Weekblad, 35: 495 (1938).

22. Starkey, R. L. Concerning the Physiology of Thiobacillus thiooxidans, and Autotrophic Bacterium Oxidizing Sulfur Under Acid Conditions. J. Bact., 10: 135 (1925).

23. __________. Isolation of Some Bacteria Which Oxidize Thiosulfate. Soil Sci., 39: 197 (1935).

24. Van Niel, C. B. On the Morphology and Physiology of the Purple and Green Sulfur Bacteria. Arch. Mikrobiol. (Ger.) 3: 1 (1931).

Sulfate Reduction and Iron Corrosion

25. Bunker, H. J. Microbiological Experiments in Anaerobic Corrosion. J. Soc. Chem. Ind. (Br.) 58 : 93 (1939).

26. Hadley, R. F. Studies in Microbiological Anaerobic Corrosion. Rpt. of the Technical Section of the Am. Gas Assn., 23 pp. (1940).

27. Pomeroy, R. Corrosion of Iron by Sulfides. Calif. Sewage Works J., 13: 31 (1941).

28. Starkey, R. L. A Study of Spore Formation and Other Morphological Characteristics of Vibrio desulfuricans. Arch. Mikrobiol. (Ger.) 9 : 268 (1938).

29. __________ Wight, K. M. Soil Areas Corrosive to Metallic Iron Through Activity of Anaerobic Sulfate-reducing Bacteria. Am. Gas Assn. Monthly, 25: 223 (1943).

30. von Wolzogen Kuhr, C. A. H. And van der Vlugt, L. S. Graphitization of Cast Iron as an Electro-biochemical Process in Anaerobic Soils. Water (Neth.) 18: 147 (1934).

Various Sulfur Transformations

31. Halvorson, H. O. Studies on the Transformations of Iron in Nature. III. The Effect of CO_2 on the Equilibrium in Iron Solutions. Soil Sci., 32: 141 (1931).

32. O'Connell, W. J., Jr. Characteristics of Microbiological Deposits in Water Circuits. Am. Petrol. Inst., Div of Refining, Eleventh Mid-year Meeting. 19 pp. (1941).

33. Starkey, R. L. And Halvorson, H. O. Studies on the Transformations of Iron in Nature. II. Concerning the Importance of Microorganisms in the Solution and Precipitation of Iron. Soil Sci., 24: 381 (1927).

Reproduced With Permission From:
TASTE AND ODOR CONTROL JOURNAL
18 (OCTOBER, 1949): 1-6

CHEMICAL COMPOSITION OF ALGAE AND ITS RELATIONSHIP TO TASTE AND ODOR*

Gerard A. Rohlich and William B. Sarles

EDITOR'S NOTE

In the June, 1949 issue of Taste and Odor Control Journal we published an article by G. A. Rohlich and W. B. Sarles entitled "The Chemistry of Organic Compounds Responsible for Tastes and Odors." The article published herewith, by the same authors, might well be considered as a companion since the two articles deal with closely related subjects. It might, therefore, be well for the reader to briefly review the subject matter in the June 1949 issue of Taste and Odor Control Journal before reading the present issue.

Editor
Taste and Odor Control Journal

Algae are known to be a cause of tastes and odors in water supplies. It has been pointed out by Whipple (1) that the living organisms can be responsible for odor. These odors may be associated with the growth of the organism and are not necessarily dependent upon death and subsequent decomposition. The odors of specific organisms have been tabulated by Whipple (see Table I). The work of Hale (2) and others has established the minimum number of units which cause perceptible levels of odor intensity.

The odors produced are odors of growth, but they may be intensified by decomposition or by destruction of the algae by chlorination, copper sulphate treatment, aeration, or other factors. The natural microbial population of the water also utilizes as nutrient material the dead algae and in the utilization produce decomposition products which may be the cause of tastes and odors.

The chemical composition of the algae has not been fully resolved into its component parts. One may generalize by saying that the cell contents of the plankton population in general is composed of proteins such as nucleo-proteins, phospho-proteins, lipo-proteins, chromo-proteins, etc., the lipids which include fats, phospho-lipids, sterols, etc., the carbohydrates, and mineral matter. In themselves these substances are not likely to produce objectionable tastes or odors. Decomposition of these materials, however, produces a great variety of compounds, many of which are capable of producing odor.

The results of the chemical analyses of various algae have been presented by Birge and Juday (3). (See Table II).

The multitude of products resulting from the decomposition of the constituent materials of the algae in water are too numerous to treat separately. Furthermore, most of the decomposition products have not been isolated and identified.

The role of the bacteria in the cycle of decomposition is not to be minimized. Whereas open water samples may be found to contain relatively few bacteria, it is known that bacteria in water will attach themselves to surfaces of solid particles, such as the cells of algae. Plankton, weed growths, and aquatic debris also provide surfaces on which bacteria will accumulate and grow.

These bacterial growths, in constant contact with the water and their source of food, consequently play an important role in the transformation of organic matter which takes place.

The presence in algae of larger percentages of protein has been noted by Fred and Peterson (4), and by Birge and Juday. Domogalla, Fred, and Peterson (5), (6), have noted the forms in which both organic and inorganic nitrogen are present in lake waters. The amino acids found by these workers are given in Table III.

It will be noted that the amino acids found are present in extremely small amounts--in the neighborhood of 0.01 ppm. for tryptophane, for example. Even the total organic nitrogen reported by these workers--in the neighborhood of 0.5 ppm.--would not provide sufficient food for rapid and extensive growth of most bacteria.

Microorganisms acting on the algae, however, will find these substances in much greater concentration. If it is assumed, as suggested by ZoBell (7) that the bacteria will become attached to the cells of the algae, and thereby decompose them following their death by more or less direct action, then the nutrition of the bacteria becomes more understandable.

---

*Published by permission of School of Public Health, University of Michigan, Ann Arbor, Michigan.

TABLE I
ODORS OF PARTICULAR ORGANISMS

Organism	*Natural Odor*
AROMATIC ODOR	
Diatomaceae	
Asterionella	Aromatic — geranium—fishy
Cyclotella	Faintly aromatic
Diatoma	Faintly aromatic
Meridion	Aromatic
Tabellaria	Aromatic — geranium—fishy
Synedra	Earthy
Protozoa	
Cryptomonas	Candied violets
Mallomonas	Aromatic—violets —fishy
GRASSY ODOR	
Cyanophyceae	
Anabaena	Grassy and moldy —green-corn—nasturtium—pigpen
Aphanizomenon	Grassy—nasturtium—pigpen
Clathrocystis	Sweet, grassy
Coelosphaerium	Sweet, grassy
Cylindrospermum	Grassy
Rivularia	Grassy and moldy
Chlorophyceae	
Dictyosphaerium	Grassy—nasturtium—fishy

Organism	*Natural Odor*
FISHY ODOR	
Diatomaceae	
Asterionella	Slightly fishy (in large numbers)
Tabellaria	Slightly fishy (in large numbers)
Chlorophyceae	
Dictyosphaerium	Faintly fishy (through chlorination)
Eudorina	Faintly fishy
Pandorina	Faintly fishy
Volvox	Fishy
Protozoa	
Bursaria	Irish moss—salt marsh—fishy
Ceratium	Vile stench (rusty brown color)
Dinobryon	Fishy, like rockweed
Glenodinium	Fishy
Mallomonas	Fishy (in large numbers)
Peridinium	Fishy, like clamshells
Synura	Ripe cucumbers—muskmelon—bitter and spicy taste
Uroglenopsis	Fishy and oily—cod-liver oil

TABLE II
CHEMICAL ANALYSES OF VARIOUS ORGANISMS

Stated in Percentages of the Dry Weight of the Sample, Ash Included
After Birge and Juday

Organisms	Nitrogen	Crude Protein (NX6.25)	Ether Extract	Pentosans	Crude Fiber	Nitrogen Free Extract	Ash	SiO_2
Cyanophyceae:								
Microcystis	8.60	53.75	4.55		2.11	32.05	7.55	0.38
Microcystis	9.25	57.94	2.67	4.97	0.26	34.82	4.31	0.13
Microcystis	6.32	39.50	2.75		0.65	52.09	5.01	
Chiefly Microcystis	8.35	52.19	5.02	7.80			7.81	1.62
Anabaena	8.27	51.69	1.11	4.81	0.63	39.40	7.17	0.95
Anabaena and Coelosphaerium	8.35	52.19	2.05	6.15	1.17	39.93	4.66	0.27
Aphanizomenon	9.30	58.12	3.72	2.04	0.53	30.12	7.51	1.16
Aphanizomenon and Anabaena	9.94	62.12	4.34	3.42	1.30	25.72	6.52	0.17
Lyngbya	9.17	57.31	2.36	5.25	3.42	31.24	5.67	0.15
Lyngbya	8.21	51.31	1.38	3.76	7.39	34.74	5.18	0.20
Diatomaceae	3.66	22.87	13.60	2.87	1.43	22.60	39.50	30.78
Chlorophyceae								
Volvox	7.61	47.56	5.54	1.00	6.32	34.30	6.28	0.24
Spirogyra	3.47	21.68	2.75	10.70	0.64	65.88	9.05	0.24
Cladophora	2.77	17.31	2.54	8.32	18.47	35.14	27.54	7.10

TABLE III
QUANTITY OF CERTAIN AMINO ACIDS FOUND IN LAKE WATERS
Stated in Milligrams per Cubic Meter

Amino Acid	Method	Mendota Surface June 18, 1924	Mendota Bottom. June 28, 1924	Devil's Lake Oct. 10, 1923	Green Lake July 18, 1923	Lake Michigan Feb. 28, 1924	Turtle Lake Jan. 18, 1924
Tryptophane	**Furth and Nobel**	10.1	13.1	12.2	14.2	5.5	8.6
Tryptophane	**Folin and Looney**	11.0	14.6	12.9	16.4	7.8	10.6
Tryptophane	**May and Rose**	9.9	12.2		16.1	6.1	
Tyrosine	**Folin and Looney**	10.4	12.5	17.6	9.6	8.3	16.7
Histidine	**Koessler and Hanke**	5.7	10.2	14.8	19.2	6.7	22.7
Cystine	**Folin and Looney**	1.5	6.1	3.3	4.4	2.1	7.5
	Total organic nitrogen	320	357	177	310	143	487

The products of bacterial decomposition of the algae may diffuse into the surrounding water and may become responsible for odors and tastes. If it is assumed that the proteins of algae are complete proteins, there will then be available for the bacteria a full complement of amino acids. Porter (8) has tabulated information available on the products formed by bacteria acting upon each of the known amino acids. Table IV presents a compilation of odorous substances which may be produced in water by bacterial action on amino acids.

In addition to the odoriferous products listed in Table IV, it is known that bacteria liberate ammonia from amino acids and, in addition, produce alcohols that possess definite odors.

The data presented deal with the proteinaceous materials which have been reported or may be found in algae. It was not possible to find in the literature accurate and complete information on the carbohydrates and fats of the algae. It may be assumed, however, that algae will contain many of the carbohydrates and fats found in other green plants. Decomposition of these materials will yield organic acids, alcohols, ketones, and esters, which may be odoriferous.

DISCUSSION AND CONCLUSIONS:

The limited amount of information which has been presented serves to bring out the necessity for research on the chemical composition of algae. Another field which appears to warrant investigation is the decomposition of algae and other plants in water by actinomycetes. For example, Umbreit and McCoy (9) have reported that up to fifty per cent of the microorganisms found in Wisconsin lake waters during the summer months are actinomycetes of the genus Micromonospora. These organisms may not only produce products different from those formed by true bacteria, but in addition, the actinomycetes themselves are known to possess a decided earthy odor. They may well be the cause of the earthy odors often reported in water supplies. It is possible that the earthy odors ascribed to the presence of diatoms may be caused by the growth of actinomycetes on the diatoms or on other algae.

An attempt has been made to indicate some of the odoriferous substances which may be formed in water by the decomposition of algae. It is possible that additional work on the chemical composition of algae will reveal the presence of odor-producing substances as yet undescribed.

In the first paper, an attempt was made to point out the multiplicity and great variety of taste- and odor-producing substances. Comparatively few of these substances have been reported to occur as the result of the decomposition of organic matter in nature. Further research in this field may well lead to the discovery of many odoriferous substances which are now detected and described solely by subjective methods.

BIBLIOGRAPHY

1. Whipple, G. C.: The Microscopy of Drinking Water. John Wiley and Sons, Inc. New York. 4th Edition (1927).

2. Hale, F. E.: Taste and Odor in the New York City's Supplies. Jour. Amer. Water Works Assoc. 10, 829-837, (1923)

3. Birge, E. A. and Juday, C.: The Inland Lakes of Wisconsin. The Plankton I. Its Quantity and Chemical Composition. Bull. No. 64, Wisconsin Geol. and Natural History Survey. p. 215. Madison (1922).

4. Fred, E. B., and Peterson, W. H.: Forms of nitrogen in pure culture of algae. The Botanical

TABLE IV

A compilation of Some of the Odoriferous Products Which May Be Produced by Bacterial Action on Amino Acids

Amino Acid	*Known Odoriferous Compound Produced*	*Odor*
glycine	methyl amine	fishy
	acetic acid	pungent
alanine	acetic acid	pungent
	propionic acid	strong, penetrating
	ethylamine	fishy
serine	formic acid acetic acid propionic acid	strong, penetrating
valine	formic and acetic acids	strong, penetrating
	isobutyric and isovaleric acids	rancid
threonine	acetic acid	see above
	butyric acid	rancid
isoleucine	methyl-ethyl propionic acid (isocaproic acid)	strong, rancid
aspartic acid	malic acid	fruity
	acetic acid	see above
	propionic acid	see above
glutamic acid	acetic acid	see above
	butyric acid	see above
phenylalanine	phenylethyl alcohol	rose
tyrosine	p-cresol and phenol	phenolic
tryptophane	skatole (β-methyl indole)	faecal
	indole	unpleasant when pure; in small quantities, jasmine
cystine	methyl mercaptan	foul
	H_2S	foul
	ethyl sulphide	garlic
cysteine	H_2S	foul
lysine	cadaverine	decaying flesh
ornithine	putridine (amino valeric)	putrid
	putrescine	putrid
proline	putridine	putrid

Gazette, 79; 324-328, (1925).

5. Peterson, S. H., Fred, E. B., and Domogalla, B.: The Occurrence of Amino Acids and Other Organic Nitrogen Compounds in Lake Water. Jour. Biol. Chem., 63, 287-295. (1925).

6. Domogalla, B. P., Fred, E. B., and Peterson, W. H.: Seasonal Variations in the Ammonia and Nitrate Content of Lake Waters. Jour. Amer. Water Works Assoc., 15, 369-385. (1926).

7. Zo Bell, C. E.: The Effect of Solid Surfaces upon Bacterial Activity. Jour. Bact. 46, 39-56. (1943).

8. Porter, J. R.: Bacterial Chemistry and Physiology. John Wiley and Sons, New York (1946).

9. Umbreit, W. W. and McCoy, E.: The Occurrence of Actinomycetes of the Genus Micromonospora in Inland Lakes: A Symposium on Hydrobiology. The University of Wisconsin Press. Madison (1941).

SEE ALSO:

Tilden, J. E.: The Algae and Their Life Relations. Univ. of Minnesota Press. Minneapolis (1935).

Smith, G. M.: Fresh Water Algae of the United States. McGraw-Hill, New York (1933).

Moncrieff, R. W.: The Chemical Senses. Leonard Hill, Ltd. London (1944). Reprinted in United States. John Wiley and Sons, Inc., New York (1946).

Reproduced With Permission From:
PUBLIC WORKS
81 (MAY 1950): 39-41, 64

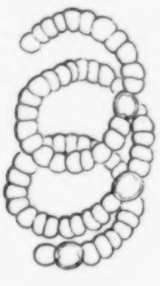
AQUATIC BIOLOGY AND THE WATER WORKS ENGINEER

James B. Lackey

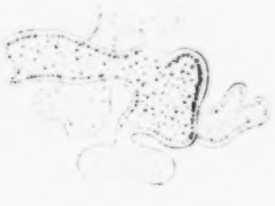
An engineer proposing to use water from the Hudson River to relieve the New York City water shortage should consider beforehand the relationship of that water's characteristics to its intended use. The same would apply to the selection of water from a river for industrial use. In either case, almost inevitably, some one characteristic of the water, or perhaps several of them, would present problems. These might be physical, and in the engineer's own sphere; or chemical, and to be dealt with by a chemist; or biological. If lucky, the engineer could obtain some help on this last problem from a bacteriologist.

Biological Problems

Of late years, an increasing number of problems, whose basis is biological, has plagued engineers in charge of water supplies. In part this increase has been due to a growing scarcity of water sources which, coupled with the demand for more water, has made it necessary to use supplies having probable undesirable characteristics; and in part it has been due to the increasing load of pollution carried by our streams. For such biological problems the engineer has few sources to which he may refer for help. Not many schools give courses in the biology of water supply; there are only two or three reference books in the field; and the number of pamphlets and professional papers available is limited. Only a few biologists have had extensive experience in this field and none of the companies manufacturing water works equipment, if the writer's memory is correct, employs a professional biologist.

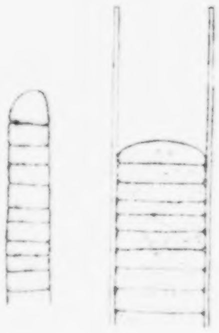


Anabaena spiroides, chain formation detail.

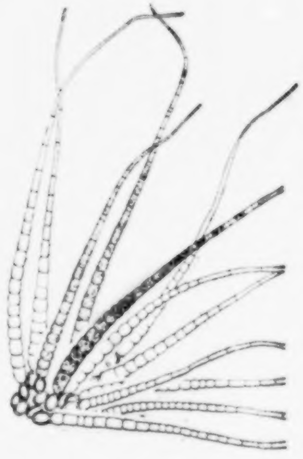


Microcystis, free-blooming and nauseous.

Broadly, the biological problems inherent in water intended for domestic or industrial use arise from (a) the natural source of the water; or (b) some source of pollution. The first of these means primarily that soft waters from granitic rocks tend to grow certain types of organisms, and these sparingly; and the harder waters from limestone rocks tend to grow other types of organisms, and in greater abundance. In a few cases, the treatment of water after it has been put into use is responsible for biological problems; for example, growths in conditioning and condenser systems.

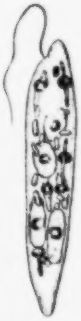


Oscillatoria, left, and Lyngbya at right.

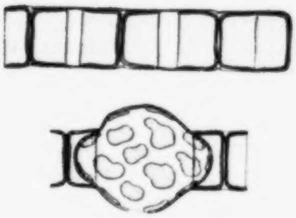


Gleotrichia, chains radiate from a center.

Classification of biological problems must necessarily be on a tentative basis, for new ones are being reported from time to time. Among the most usual problems are tastes and odors due to algae or protozoa. Soft waters tend to develop great accumulations of particular species of a few types which produce nauseous tastes and odors. These accumulations, termed blooms, usually consist of a single species which, within a few days, reaches a high population peak and then declines. These blooms fortunately rarely persist, and usually a given species blooms only once a year. Most of these soft water (pH below 6.8) forms belong to the yellow brown or brown flagellated algae. Some are pictured herewith.

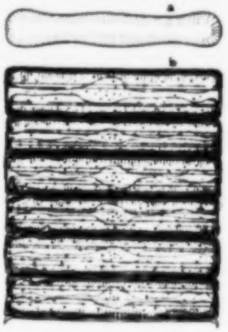


Euglena gracilis favors fertilized water.

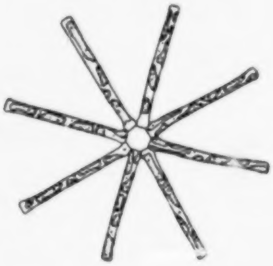


Melosira varians; auxospore shown below.

The cause of such blooms are still unknown, despite considerable research; but Sawyer and Lackey were unable to find blooms until nitrate nitrogen (NO_3) and phosphate phosphorus (PO_4) accumulated in natural waters to respective values of 0.30 and 0.015 ppm or higher. These represent threshold values. Any source contributing these materials to a water should warn the engineer to look for possible trouble from biologic growths.



Fragellaria, showing top and side views.



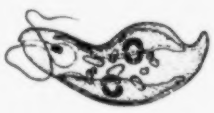
Asterionella, troublesome for taste and odor.

Fertilization

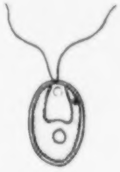
It has lately become common practice to "fertilize" ponds for biologic growths and to increase fish production with commercial fertilizers. Dr. Loosanoff of the Fish and Wildlife Laboratory at Milford, Connecticut, which studies the production of oysters, clams and mussels, raises food for them as a dense soup of green algae by adding commercial fertilizer to water in a large vat. Food requirements are basic for biologic growths; and, after them, temperature, light effects and other factors are important in bloom production.

Natural waters may be subject to heavy increases in fertility from such causes as domestic sewage, industrial wastes, or fertilizer from cultivated or pasture lands.

Water supplies which come from springs, artesian wells or pumped wells usually offer few biological troubles, though springs in limestone regions are always suspect so far as coliform counts are concerned. The other two sources sometimes suffer from iron content or deposits in the pipes. Iron troubles are frequently attributed to iron bacteria, supposedly capable of using ionic iron and converting it to insoluble salts. It should be pointed out that little proof of this can be adduced; that a recent paper by Pringsheim does not support this; and that the bulk of evidence points to such troubles as being chemical or electrical.

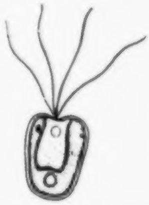


Euglena pisciformis, found in polluted water.

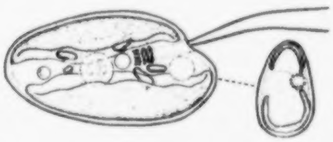


Chlamydomonas, with with two flagella.

Perhaps the best water should be that from a reservoir whose catchment area is heavily wooded and largely uninhabited. Such supplies face trouble from color whose biological basis is the leaf fall from deciduous trees. A watershed whose cover is pine or spruce will accumulate leaf debris at a much slower rate. In either case, the leaf debris is subject to slow biological decomposition and this, plus the nitrates and phosphates contributed by surface runoff, may eventually accumulate fertilizer to a threshold value for algal blooms. There is no escape from at least an occasional bloom in any stored surface water.

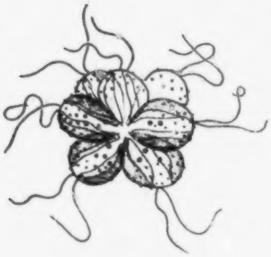


Couteria, relatively innocuous.

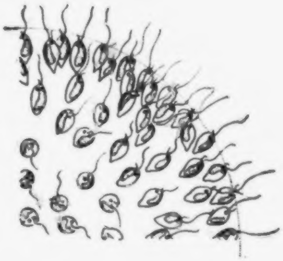


Cryptomonas; entire animal shown at left.

Watershed forests are sometimes subject to heavy insect infestation. Control of such insects (example, spruce budworm) by spraying or dusting should be undertaken with great care. A heavy rain closely following a heavy application of insect killer might possible contribute dangerously to the water.



Synura colony.

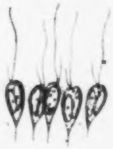


Uroglenopsis may cause taste and odor.

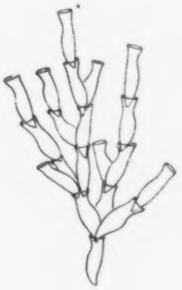
Other Sources of Trouble

In a soft water reservoir the troubles resulting from heavy biological growths may be taste and odor from Ceratium, Uroglena, Dinobryon and Synura, but there is little likelihood that these forms will clog filters. Soft water rivers are not apt to be sources of serious trouble, though there are a few diatoms which may cause occasional filter clogging, virtually overnight, or which may cause tastes and odors.

Major troubles occur in hard water streams in densely populated or rich agricultural regions. These streams usually have a heavy plankton content. For a part of the year, such growths may be controlled by nature. Winter and spring floods carrying silt are destructive to plackton; low temperatures lengthen the time of reproduction; and decreased hours of light have an adverse effect. However, the engineer must recognize that biological growths obey the same laws as the vegetables and weeds in his garden. Peas prefer cool weather. So do most of the diatoms, and the yellow green or yellow brown flagellated algae. Therefore troubles may occur in cool as well as in warm weather.



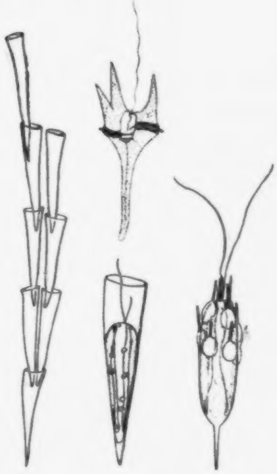
Uroglenopsis, showing individual cells.



Dinobryon, yellow brown flagellate.

From a hard water reservoir, diatoms such as Synedra, Melosira or Asterionella; the yellow brown flagellate Mallomas; the green flagellates Chlamydomonas, Platymonas and Euglena; the olive green flagellate Cryptomonas; and occasionally the green non-flagellated algae such as Dictyosphaerum may cause trouble. Some of these may occur at any time of the year; they may cause tastes and odors; they clog filters; and they may support an active growth on the filters. Hard water reservoirs, especially if warm shallow lakes, also are subject to nauseous blooms of blue green algae such as Anabaena, Microcystis, Lyngbya or Clestrichia, and these can be just as objectionable.

Hard water rivers are often literally green, olive green or brown in late summer, with microscopic biologic growths. The writer and a U. S. Public Health Service Sanitary Engineer, the late H. W. Kehr, once computed the Scioto River water at a certain station contained 30,000 ppm of living plankton. The potential BOD of such a water, its possibilities as a bacterial substrate on a filter bed, its reaction to coagulation, its filter clogging potential, and its possible taste and odor causing ability should make an engineer speculate that there surely must be an easier supply somewhere. Nor is this an isolated case--other rivers in Ohio, Indiana, Illinois and Wisconsin have shown equally high counts. In most cases, no single organism is implicated alone, but the aggregate of organisms cause trouble. Once in a while some single organism as Euglena polymorpha or Cryptomonas erosa is at fault.



Ceratium, top center; Dinobryon at left and center; Synura, single cell, at bottom right.

Fertilization and topography are primary factors. For example, the East fork of the Kentucky Licking River drains a timbered, sparsely populated area with little arable land, and that not well fertilized. The West fork drains the rich bluegrass country. The plankton content of the West fork is many times that of the other. Because its gradient is steep, a small rain on the East fork sends an abrasive slug of silt down the river, scouring it almost clean of plankton. The small population left must then begin anew to rebuild. A much heavier rain is needed to achieve the same result on the West fork.

The Effect of Sewage

The sewage of man and his domestic animals is a potent fertilizer. The increase of plankton in the Scioto River of Ohio, below the outfall of the Columbus sewage treatment plant, was shown to be very great. Possibly it is not always realized that the richest source of nitrogen in sewage is the urine which passes at once through a treatment plant. The contributions of phosphates by such plants are also large. In worrying about the organic load and BOD contributed by sewage and trade wastes, we would be wise not to forget about the inorganic, soluble fertilizer from the same sources.

Biologic growths in lakes arise from the same general sources. Lake Mendota in Wisconsin, receiving negligible agricultural or human drainage, gave trouble once in two years of study; but neighboring Lakes Monoma, Waubesa, and Kegonsa, more heavily fertilized, especially by the city of Madison and its industries, provide enough trouble to be a source of legislative action.

Industrial wastes are diverse in biologic action. Some produce heavy growths of deleterious organisms-- any waste having a high BOD belongs in this category. For example, distillery wastes produce enormous quantities of bacteria and paper mill wastes tend to produce heavy growths of Sphaerotilus. Others-- chromium wastes, leachings from exposed coal seams and steel mill pickle wastes-- are extremely toxic. The biologic principle involved is an upset of biologic balance and any such upset is almost invariably bad. If we load a stream with sulfur-containing matter, we can expect a growth of H_2S producing bacteria and resultant outcry from people dwelling along the banks.

Advance knowledge of the probable biologic effects is wise and often profitable, for rectification may be costly or impossible. We no longer overload a stream with raw sewage, but is it much wiser to fertilize it so heavily that it produces microscopic weeds? Or to utilize a stream for water supply, whose plankton content is so high as to make it very expensive or almost impossible to treat for taste, odor or color?

There are distinctly bad aspects to the presence of profuse growths of algae, and it would be wise to investigate other supplies less liable to their occurrence. Though many new organic chemicals are being developed to kill specific growths such as poison ivy, water chestnut, diatoms, gnats or even fish, it should be ascertained that they are harmless to other organisms before they are used or they may cause as much trouble, by upsetting the stream balance as they accomplish good.

Where the Aquatic Biologist Can Help

Before a source for a water supply is finally selected, it should be surveyed by a competent aquatic biologist in order to evaluate present and probable future biological conditions tending to affect the quality of the water. Reservoirs and storage facility plans should be reviewed by him to determine possible changes. Such work as the elimination of undesirable shallow areas can often be accomplished more cheaply during construction than these areas can be maintained after the reservoir is filled.

In areas where blooms or heavy growths of aquatic plants present an annual problem, a study by a qualified aquatic biologist may indicate the causes for such growths and also methods of relief. If excessive growths occur and something must be done in a hurry, as is so often the case, the engineer or superintendent may call on a biologist from the nearest college or university. It may be too late at this stage for remedial measure, but identification of the growth and determination of the reasons for it may help in the future.

Reproduced With Permission From:

TASTE AND ODOR CONTROL JOURNAL
17 (JUNE, 1951): 1-8

PRE-TREATMENT BASIN FOR ALGAE REMOVAL

Andrew J. Marx, Water Plant Engineer,

Menasha Electric and Water Utilities, Menasha, Wis.

Winnebago is an Indian word which means "stinking or smelly water," so even back in the days of the Indians, Lake Winnebago must have been much the same as it is now.

For those of you who are not familiar with the lake, it is about 30 miles long and 10 miles wide, but unfortunately, the deepest spot in the lake is only 21 feet, and the average depth is considerably less than that. Water temperatures of 70 to 80 degrees are common all summer, and one day during the summer of 1936 we recorded a temperature of

92 in the raw water entering our plant at Menasha.

The lake is supplied and kept filled by flowage from the upper Fox and Wolf rivers and their tributaries, which are the drainage ditches for thousands of acres of farmland in central and northern Wisconsin. With this drainage is included a rich supply of nitrates and phosphates. When this is added to the high water temperatures and 300 square miles of surface for the action of sunlight, it is no wonder there are severe periodic growths of algae every week or two, from late spring until early fall.

This lake, filled with algae as it is, is the one and only supply of raw water for the cities of Oshkosh, Neenah, Menasha, and Appleton.

The intensity of Algae growth varies considerably from year to year, and during different times of the year. Growths got worse until they reached a peak in 1939, and then gradually tapered off. 1943 to 1946 was a very good period. 1948 and 1949 were worse than average, and this year was very good again. This was probably due to a lot of rain and cold weather, and steady winds.

As an example of how bad the water can get, during August of 1939 the water temperature was 85, the algae count reached 50,000 units per ml., turbidity was 200 ppm, and the odor reached 300. A treatment of over 100 ppm of carbon and 9 grains of alum, cut the odor down to about 15 and the turbidity down to about 10, in the finished water. This water was obviously not satisfactory for use, but nothing could be done about it. The Neenah plant was turning out the same kind of water on the same day, and a representative of the State Board of Health was there to try to help us, although nothing could be done. Our cost for chemicals alone for the month of August, 1939, was 7¢ per thousand gallons. Of course, this was an extreme case, and many summers have been very good, but the potential possibility for these extremes is there all the time.

After the summer of 1939 it was obvious that something had to be done, so during 1940 an International Accelator and an Ozonator plant were set up at Appleton, and a Permutit Precipitator at Menasha. Every type of coagulant and odor removal material were tried, with settling in different sequences before and after the up-flow units. These units were found to be excellent for softening, but they were no better than the conventional plant for removal of algae and odor.

About this time we began to think about the use of Copper Sulphate, which had been used in many places for years for algae control. The trouble was that there was no place to use it. It would be ridiculous to think of trying to treat Lake Winnebago, even if all the cities in the valley got together, because of the extremely large surface area. Use of Copper Sulphate right in the plant process gave no improvement either, because it requires several days instead of several hours to get results with this chemical.

The next thought was the possibility of making a small lake of our own which could be filled and treated, and, and then used after several days of standing. This would have required two separate basins, and a great expenditure of money. This led to the idea of using continuous treatment in only one basin, taking in polluted water at the inlet, letting the water gradually work to the other end of the basin, and drawing clear water off at the outlet.

No data could be found on any treatment of this kind for any water plant in the country. Many plants get water from reservoirs where fairly clear water runs into them. When algae begin to grow in the reservoirs the surface is treated before the growths get very severe. The same is done in small lakes which supply many plants. However, in our case, we would be taking in water which at times would already be grossly polluted with algae, with no control over it, and all of which would have to be killed by continuous treatment at the inlet.

To find out whether this would be practical, we set up a small pilot plant on the roof of our settling basin. Because of the difficulty of very small flows, we treated batches of water every day and let them stand in small tanks. Daily records were made of weather, odor, turbidity, and algae counts. Results in general were almost unbelievable in the amount of odor reduction and the saving in activated carbon, which results indicated. Good results were obtained in anywhere from three to seven days, depending on the weather; large amounts of heat and sunlight bringing good results in three days, while cloudy, cool days required six to seven days of standing.

These results made it look as if a basin on this type would be practical, if a suitable place could be found to build one, and if it could be made large enough so that the water would be in the basin for at least seven days after treatment at the inlet. Also it would have to be near enough to the plant, so the cost of a pipe line to the plant would not be prohibitive.

What looked like an ideal spot was found in a triangular shaped swamp on the eastern side of the city, about half a mile from the water plant. This swamp belonged to a member of the Water and Light Commission, and was given to the City of Menasha for this purpose.

The engineering firm of Orbison and Orbison at Appleton was then hired for the purpose of surveying and laying out the site; and drawing up plans and specifications for digging out the basin to the proper depth, constructing baffles, building a chemical feed house, and laying a pipe line to the water plant. After taking bids, the contract for construction of the basin was given to Furton Bros. of Menominee,

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

View of Pre-treatment Basin, looking northeast. Note concrete baffles.

Michigan.

Work was soon begun, but many unforeseen difficulties seemed to make the job more difficult than the contractor anticipated. To add to the trouble, the United States was just getting into full swing in war production, with the attendant cuts in civilian production. In a few months most of the contractor's trucks were standing alongside the road, broken down, with no tires or parts available. When the job was about 7/8 completed, the contractor ended up by going bankrupt. A series of court cases began then, in 1943, and most of it ended in 1946, with a decision in favor of the city.

During this time the basin stood idle, even though it was completed enough so it could have been used. The incomplete part was mostly rip-rap on the outer walls, and more depth in one section. The basin was put in operation in the spring of 1946, and has been used regularly since that time during the algae season, and the last two years it has been used to advantage all year, both summer and winter.

The construction and layout of the basin is as follows: (See Fig. I)

Water from the lake at the mouth of the Fox River flows through a channel about 1000 feet long and 50 feet wide, and a depth of several feet, to the entrance of the basin. This channel has been in existance for many years, and was dug originally for boats to get to one of the former beaches in Menasha, as the lake was too shallow for large boats to get near the shore.

Water from this channel then goes through a large screen for removing coarse material, travels through a pipe under the chemical feed house, and

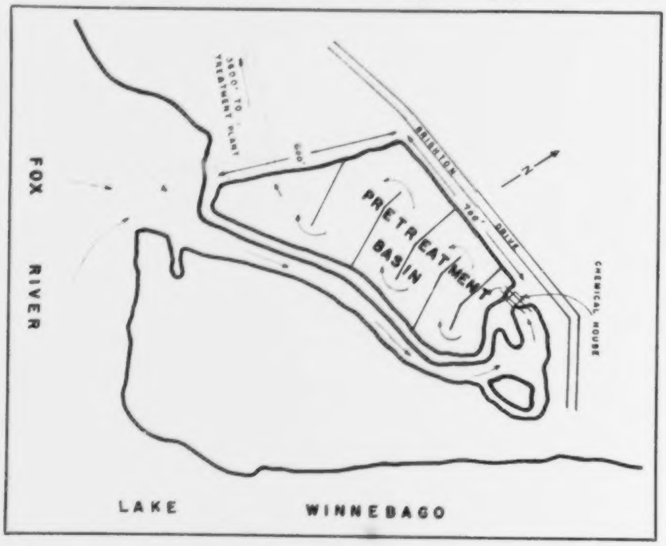


Figure 1

then into the basin proper. It is at this point that copper sulphate is fed into the water continuously during the algae season.

The chemical feed house is a small brick building about 8 by 10 feet, which contains a 1000 gallon wooden tank, and a small liquid feeder. Copper sulphate is dissolved in this tank at the rate of one pound to two gallons of water. A quart of sulphuric acid is also added to each tankful of solution to keep the solution clear. The tank is connected to a small liquid feeder which meters the solution and adds it to the raw water as it enters the basin. A tankful of this solution lasts a week.

The basin proper is roughly triangular in shape, with the longest side 1000 feet, and the other two sides both about 700 feet. The only reason for this shape is that the land available happened to be shaped this way. Cement baffles, alternating from side to side, run about 2/3 of the distance across the basin. They are set on a good footing below the bottom of the basin, and extend several inches above the high water level. These baffles force the water from one shore of the basin to the other five times as it travels from the inlet to the outlet, preventing by-passing and making full use of the entire basin. All the walls and the bottom of the basin are of clay, or whatever material was there originally. The depth of the basin, except near the edges, is seven feet at normal water level. The estimated capacity is about 25 million gallons, which gives us a retention period of seven or eight days at the present time.

The basin is connected to the water plant by about 3600 feet of corrugated iron pipe, 30 inches in diameter similar to the pipe used in culverts. This pipe has shown a resistance to flow considerably higher than expected, so that at some time in the future water may have to be pumped through it, instead of flowing by gravity. No pumping is done anywhere at the present time. Water flows from the river through the channel, through the basin, and then through the pipe line, all by gravity. The pipe line enters our old river intake at a point about 100 feet behind the plant. In an emergency of any kind we can still get water directly from the river by merely opening and closing the proper valves.

Now let us follow the water on its circuit through the basin. It leaves the river and flows through the channel leading to the basin, taking about three days to get there from the river. As the channel has very little flow, and is protected from wind, much of the algae in the water comes to the top, and begins to decompose even before treatment in the basin. Logs used as a skimming device in front of the chemical feed house, keep much of the floating algae from entering the basin. Also the short pipes leading from the channel to the basin are at the bottom of the water, so very little surface water is taken into the basin.

As the water flows into the basin it is given a dosage of 2 ppm of copper sulphate. This same dosage is fed continously throughout the algae season, regardless of the type of algae, pumpage, or any other variable. After treatment, all of the algae is killed, and much of it comes to the surface, looking bluish-white. This color is always an indication that the algae is being killed successfully. At this point the water is very unsatisfactory, odors being at least twice as high as they were before, and the appearance of the water is very unsatisfactory to anyone looking at it.

As the water goes farther along in the basin, much of the algae on the surface begins to disappear. It is important to realize that the algae is disappearing by decomposition, not by settling. The algae does not go to the bottom of the basin, but disintegrates into very small pieces, and much of it decomposes completely. It is this process of decomposition which is hastened by sunlight and warm weather, and retarded by cold, rainy weather. As algae grows best with heat and sunlight, and the basin also works best then, results are always very good.

As the water gets to the middle of the basin, the only thing left is a yellow, oily scum on the surface of the water. At this point the odor is considerably below that of the raw water. By the time the water reaches the outlet, all the scum is gone, and just a very fine turbidity is left. From there the water is ready to go to the water plant for its regular treatment with chlorine, carbon, alum, and chlorine dioxide.

No trouble has ever been experienced in killing algae with a dosage of 2 ppm of copper sulphate. Diatoms which appear in the spring and fall are easily killed. Some of the green algae which appear for a short time after that are difficult to kill, but very few green algae grow in Winnebago, because of the early growth and predominance of the blue-greens, which take over and grow heavily all summer. These are also very easy to kill with small dosages of copper sulphate. Undoubtedly at times less copper sulphate could be used, but the extra testing and watching, and the possibility of under treatment, would not be a worth-while saving. As it is, the cost per million gallons is only about $1.50.

Now, what is the basin doing in the way of odor reduction? An accurate survey was made during the algae season in 1949, which was an average year, or perhaps a little worse than average. I am not going to read off endless figures and facts, which are tiresome and hard to digest. I have just a few to show our results. The average odor in the water entering the basin was 50, the maximum during the season was 100, and the minimum during the season was 20. The average odor on the water leaving the basin was 16 instead of 50, the maximum was 25 instead of 100, and the minimum was 10 instead of 20. You will note that the reduction, based on

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

View of Chemical Feed House, looking southwest from stream leading to the basin.

both averages, was 67%. While this seems very good, it is even better than it seems. While the odor of fresh algae from the lake is very difficult to remove, and is greatly increased or aggravated by chlorination, the odor from the basin is very easy to remove, and is cut down below half by chlorination. The odor is classed as a hydrogen sulfide odor, and most of the time, after chlorination, the odor intensity is below five. The most obvious thing to us is that, while the odor of the lake water varies a great deal from day to day, the odor of the water from the basin after chlorination never varies. As a result, instead of continually changing alum and carbon dosages, and trying to out-guess nature, we can set chemical feeds at a very minimum point, and make only very slight changes all summer.

To find out how much these odor reductions make in the amount of carbon needed, I have a few more figures I would like to read. It is very easy to give figures on the amount of chemicals required by basin water, because that is what we are using, but figuring the amount needed for lake water, which we are not using, is a different matter. The best that can be done is to make a rough estimate of how much might have been used, based on lake odor tests and past experience, and combining these with figures on the actual amounts used in Oshkosh, Neenah and Appleton, who are using lake water. The figures arrived at in this way for the period of June 1 to Sept. 30, 1949, are 88,000 pounds of carbon for lake water. We actually used only 13,000 pounds during this same period on our treated basin water. This amount was only 1/5 of the amount that we would likely have had to use on lake water, to turn out water of the same quality. In actual dollars and cents it is a saving of about $6000.00, with carbon at 8¢ per pound.

The saving in alum is much harder to estimate, and is not nearly as high as the carbon saving, and was not expected to be. A rough estimate would be 1/3 less during the algae season in an average year, which would be 40,000 pounds or $600.00, which would just about pay for the copper sulphate used.

Treatment at the water plant is very simple since the reservoir has been in operation. At the beginning of the algae season, the carbon feeder is started and set to feed about 4 ppm, and is left at this point all summer. This small amount removes most of the odor remaining in the water from the basin, and also stabilizes sludge in the settling basins in the plant, as we have no automatic sludge removal.

Besides the savings in chemicals and greatly simplified treatment, the basin also gave us several unexpected benefits:

Every spring, after the first heavy rain, the turbidity in the river used to go to two or three hundred, and every severe storm used to do the same thing. The basin is at the mouth of the river where the water stays clear, and we have had no attacks of high turbidity since the basin has been in operation.

This also applies to weeds. Each spring and fall, especially just after high winds, our intake used to get covered with weeds, so that at times we had trouble getting enough water into the plant. We have had no weed trouble since, as weeds either float or settle in the channel leading to the basin, or stop harmlessly at the screen at the entrance to the basin, where they can be removed.

The most pleasing result we got unexpectidly from the basin is the complete elimination of anchor ice. Some of you may have it and some may not, but it certainly is a headache. It always closed our intake one or two nights in early winter, for two or three hours. It always happens the first real cold night of the winter, with a north wind blowing and a temperature of ten to twenty degrees above zero. Wind and current in the river prevented the water from freezing, and as a result the water super cooled and stuck to the iron intake, which would be below the freezing point. We have had a 24'' intake, wide open at the end, close up solid with ice. However, in the basin, the surface is protected from wind by shoreline and baffles, and there is no current, so the water freezes over the surface the instant it is ready to freeze, thus eliminating the cause of anchor ice.

The last advantage we have noticed is the sharp reduction in Coliform organisms. For example, during the month of August, 1949, the average index in the Fox River at Menasha, and also at the Neenah plant, was about 500. The average index in the water coming from our basin during the same period was 30 instead of 500. On several days the index was zero. As copper sulphate should have little effect on bacteria, this drop must be due to additional standing and additional exposure to sunlight.

Now I have a list of questions which are always asked about the basin:

How are we going to clean the bottom?

As I mentioned earlier in this paper, algae decompose and do not settle to the bottom. In five years of use, less than an inch of material has settled on the bottom of the basin, and most of this is material which has washed away from the sides. If it ever needs cleaning, it will be a good many years from now.

Are there weeds and fish in the basin?

There are no weeds in the basin now. There were the first year, but they soon disintegrated after treatment was begun. Since that time none have been able to grow. There are a lot of fish of all kinds in the basin, and the copper sulphate apparently does not hurt them, as very few dead fish are ever found. At one time a school of carp were in the end section of the basin, stirring up the water and making it turbid, so we tried to kill them by an application of 20 ppm of copper sulphate right over the top of the fish. This is about four times the tolerance given for carp to copper sulphate. We watched them play around as happily as ever after that, and not one fish died.

How much copper stays in the water?

As the raw water is quite alkaline, the copper precipitates as the hydroxide a few minutes after it is added, and thus the copper is soon out of solution. Even when a high dosage was used to try to kill the fish, only a trace remained in solution after a few hours.

Do the algae begin growing again near the outlet of the basin?

In the five years the basin has been in use, not one live algae unit has ever been found in the water coming to the plant, and no sign of an aftergrowth has ever been noted in any part of the basin. Apparently a week is not time enough for another growth to start, after one growth has been stopped. Occasionally protozoa or slightly larger animals are found, as copper sulphate does not kill them, but they do not do any harm.

In conclusion, I would like to say that after five years of use, our basin has more than proven its worth in large chemical savings, greatly simplified treatment, and insurance against having to turn out unsatisfactory finished water during severe algae growths. It has definitely solved the water problem for the City of Menasha.

Reproduced With Permission From:
TASTE AND ODOR CONTROL
IN WATER PURIFICATION. 1955
WEST VIRGINIA PULP AND PAPER CO., N.Y.
pp 17-21

INDUSTRIAL WASTES AS A SOURCE OF TASTES AND ODORS IN WATER SUPPLIES

A. M. Buswell, Chief, Illinois Water Survey, and
Professor of Chemistry, University of Illinois, Urbana, Ill.

The senses of taste and smell differ radically from the other three--sight, sound and detection of heat. These last three senses are simulated by radiations or waves whose intensity and frequency define the respective sensations accurately. Certain wavelengths in the visible spectrum produce the sensation of color, and a photoelectric cell determines its intensity or brightness. Infrared radiations produce the sensation of heat and a thermocouple registers intensity. Sound is described in terms of pitch and decibels. But neither tastes nor odors can be measured by any physical unit.

Accurately speaking there are only four tastes: sour, salt, sweet, and bitter. Other apparent tastes or flavors result from the fact that an observer ordinarily smells anything he tastes.

The quantitative unit of odor is the olfacty which is the minimum detectable number of grams of the substance per cubic centimeter divided by its molecular weight and multiplied by Avogadro's number (6.06×10^{23}). It is, thus, the number of molecules per cubic centimeter which can be detected.

The olfacties of some common substances are as follows:

Ethyl alcohol	30×10^{14}
Benzene	41×10^{12}
Phenol	26×10^{12}
Cresol	50×10^{11}
Pyridine	31×10^{10}
Iodoform	42×10^{9}
Skatol	17×10^{8}

Iodoform, which is similar to the chlorophenols, is seen to have a very low olfacty number. Some 4,000 different kinds of odors are reported, but their classification is almost entirely dependent on the individual observer and it is seldom that any two individuals agree in their qualitative groupings. The chemical groups, which are supposed to be responsible for the odorous characteristics of the molecule as given in Volume I of the International Critical Tables,[*] are: esters; aldehydes; ketones; ethers; alcohols; acids; nitrites; nitriles; terpenes; pinenes; sulfides; arsenides; cacodyls; halogens; pyridine; pyrrole.

One might expect some correlation between these odoriphore groups and odor but no very consistent agreement has been noted. One point is important and that is that odoriphore groups are, in general, reduced substances which are easily oxidized. This fact is of practical importance. It indicates that organic matter which putresces and produces reducing conditions may generate odorous compounds.

From the list of odoriphore groups given above one can see that practically every manufacturing process may give rise to odors. A suggested classification of industries which have been known to cause odors in water supplies is given below.

I. Industries producing organic wastes
  A. Manufacturers of organic products
    1. Organic chemicals--dyes, medicinals, plastics, etc.
    2. Coke (quench water)
    3. Ammonia recovery (gas liquor)
    4. Wood oil (charcoal) phenols, cresols, acids
    5. Tar
    6. Petroleum products--gasoline, crude oil, road oil, etc.

  B. Processors of natural products
    1. Foods and beverages
      a. Vegetable products
        (1) Canned goods (wash water, waste juices, cooking liquor)
        (2) Starches--corn (steep liquor, sulfur, nitrogen); potato (wash water, pulp)
        (3) Sauerkraut (acid, very foul)
        (4) Citrus fruit (pulp)
        (5) Distilled beverages (nitrogenous and lower sugars, acids)
        (6) Yeast (nitrogenous and lower sugars, acids)
        (7) Sugar beets (nitrogenous and lower sugars, acids)
      b. Animal products
        (1) Meats--slaughtering, rendering, packing (nitrogenous, putrescible)
        (2) Fish (amines)
        (3) Gelatin
        (4) Dairy foods (nitrogenous, foul acid)
    2. Textiles (washing soap, sulfates, grease, synthetic detergents)
    3. Leather (nitrogenous, foul)
    4. Paper--sulfite pulp (lignin, acids); conversion mills (paper odor)
    5. Strawboard

II. Industries producing inorganic wastes
  A. Industries producing true tastes--salty, sour, or bitter
    1. Petroleum products (salt from oil brines)
    2. Steel (copperas from pickle liquors)
    3. Phosphate (acid)
  B. Industries producing odors--Chemicals--cyanides, sulfides, sulfates (indirect by reduction), ammonia and ammonium sulfides (gas liquor)

Before discussing these industrial sources it should be pointed out that odors may arise in three ways: First, the waste may contain some substance of relatively low olfacty like the phenols or pyridine. This is true of all of the wastes produced by manufacturers of organic products and of some produced by processors of natural products. Second, the waste may contain substances which, in the absence of oxygen, decompose and produce substances of low olfacty numbers. This applies particularly to all of the wastes in the food and beverage group. Third, the waste may contain substances which react with constituents in the water to produce substances of low olfacty numbers. Examples are the phenolic substances which react with chlorine to form chlorophenols, and the reduction of sulfates to form hydrogen sulfide and mercaptans.

---

[*] McGraw-Hill Publishing Co., New York (1928).

A detailed discussion of industrial wastes as sources of odor would require more space than can be allotted. For example, in making a study of a very troublesome odor problem, the author located seventeen distinct points in one manufacturing plant where highly odorous substances were discharged. If the reader will allow a reasonable tolerance in taking exceptions, the following generalizations will be found helpful.

The serious sources of odor are almost exclusively organic substances. Only one inorganic substance of low olfacty number--osmic acid--is to be found in a list of 100 odor-producing compounds given in International Critical Tables, and osmic acid is not likely to be discharged into a water supply. The phenolic substances, class I-A, characteristically produce wastes which can be detected directly in very high dilutions, 10 parts per billion being the lowest dilution in which phenol can usually be detected in water. Other phenolic substances are detectable in even lower concentrations. Wastes from this class of industry do not decompose readily and when decomposed by bacterial action the odor condition is improved rather than aggravated. Phenolic bodies react with chlorine, however, forming substances detectable in even lower concentrations, sometimes as low as 1 part per billion.

A newcomer among taste- and odor-producing wastes is the synthetic detergent. The substances falling under this head are somewhat diverse in chemical composition, but are all composed of rather large organic molecules. Many of them contain cyclic groups of the aromatic series which characteristically produce strong odors when chlorinated. Others contain amines which have characteristic soapy odors. In general, the substances produce a detectable soapy odor and taste in dilutions of 5 parts per million. Chlorination intensifies the odor.

The wastes resulting from the processing of natural products have been divided into two classes--those resulting from food and beverage production and those from the manufacture of textiles. Those resulting from the manufacture of foods and beverages commonly contain substances which decompose or putresce very readily. The material as discharged ordinarily does not carry highly odorous substances, but the decomposition products are usually exceedingly foul, being detectable frequently in low concentrations, as skatol, amines, and sulfur compounds.

The third method in which odors may arise--i.e., in reaction with materials in the water--is frequently overlooked. Sulfate waters are most troublesome in this respect. When the sulfate content of the water is high a relatively small amount of inoffensive organic matter of a cellulose or carbohydrate composition, free from sulfur and nitrogen, may produce odors of hydrogen sulfide and other sulfur compounds when no odor would result if the same effluent were discharged in a low sulfate water.

Some of the wastes mentioned above may produce true tastes, particularly bitter substances. The inorganic wastes, notably brines and by-products from crude oil production or the manufacture of inorganic chemicals, sometimes produce salty tastes in water. The tolerance in this respect is approximately 500 parts per million, although some natural waters contain more minerals than that. Mineral wastes have an important relation to water in that they frequently contain sulfates, which, in the presence of even small amounts of organic matter, may be reduced to form hydrogen sulfide and organic compounds.

Manufacturers have done much to reduce the quantity of wastes they discharge into the sources of water supply, and more and more effort is being expended along that line. Even at best, however, it is doubtful whether industry can or should completely eliminate this type of waste disposal. The water treatment plant can most economically handle the last fractions of this load and should be prepared to do so.

Chapter VII

BIOLOGY OF SEWAGE AND INDUSTRIAL WASTES TREATMENT

Reproduced With Permission From:
JOURNAL OF MILK AND FOOD TECHNOLOGY
11(1948): 293-300

THE CHEMISTRY AND BIOLOGY OF MILK WASTE DISPOSAL*

Theodore F. Wisniewski

Senior Assistant Sanitary Engineer
State Board of Health, Madison, Wisconsin

Milk, a mixture of proteins, fats, sugars, and salts containing calcium, phosphorus, iron, copper, and magnesium, all of which are essential to maintenance of life processes, is often termed as a nearly perfect food for humans. When milk is processed a certain percentage is lost to sewers and is discharged to streams or treatment plants where it is an excellent food for bacteria that break down complex organic materials into simpler, more stable, and fairly innocuous substances.

Modern methods for disposal of milk wastes are a combination of devices for confining in a small space all the physical, chemical, and biological processes that otherwise might be spread over a long stretch of receiving streams. Microorganisms play an important part in intermittent sand filters, high and low rate trickling filters, the activated sludge process, and in sludge digestion.

These microorganisms, regardless of size, all have common requirements that must be met in order for them to remain alive. Food must be of a suitable type and of adequate supply, there must be sufficient oxygen, proper temperature range, and a suitable medium in which to live. All of these conditions are normally provided by the milk wastes in a properly functioning treatment plant. The organic waste constituents include the three classes of food: carbohydrates, proteins, and fats, plus their decomposition products. The inorganic compounds include mineral salts ordinarily found in water plus ammonium salts, nitrates, and nitrites. Some waste matters are carried in suspension, others in solution, and still others in a finely divided colloidal state. The presence of these foods and minerals qualify milk wastes as a suitable culture medium capable of supporting multitudes of various microorganisms. Of these, bacteria are the most predominant both in numbers and in extent of activity.

Milk wastes contain acid forming, alkali forming, and proteolytic bacteria all of which are active in the stabilization of the wastes.

The efficiency of bacteria in reducing organic matter lies in their ability to produce a variety of enzymes, the complicated protein compounds that bring about digestion without being used up in the process. These are specific in action. Carbohydrases act only on carbohydrates, proteases only on proteins, and lipases only on fats. The result of digestion here is the same as in the digestive apparatus of man. Compounds are reduced to soluble form or made chemically more simple. Bacterial enzymes may be external or internal. External ones are liberated from the bacterial cells and diffuse into the wastes, transforming organic substances so that they may pass through bacterial cell membranes. See Figure 1. This is important since it is the only method by which saprophytes may obtain food. Internal enzymes are retained by the bacterial cell and are effective in the release of energy from the digested food. Energy is required in such vital processes as growth, reproduction, and movement. Energy release is brought about by action of oxygen upon the food contained in the wastes. It is the oxidation reaction called respiration. Most organisms require molecular oxygen for this activity with bacteria extracting it from the wastes in which they are immersed. Biochemical oxygen demand (B.O.D.) is a measure of the degree to which they are able to extract oxygen dissolved in the medium for action upon the available food supply. Organisms functioning in the presence of free oxygen are spoken of as aerobes.

Certain other bacteria are able to function where the free oxygen concentration is zero provided suitable compounds containing oxygen are available. These compounds are reduced by the bacteria in order to obtain oxygen to apply to food. Such bacteria are called anaerobes and function primarily in sludge digestion.

TRICKLING FILTER

The trickling filter is a device for providing and

---

* Presented at the Thirty-fourth Annual Meeting of the International Association of Milk and Food Sanitarians, Inc., Milwaukee, Wis., October 17, 1947.

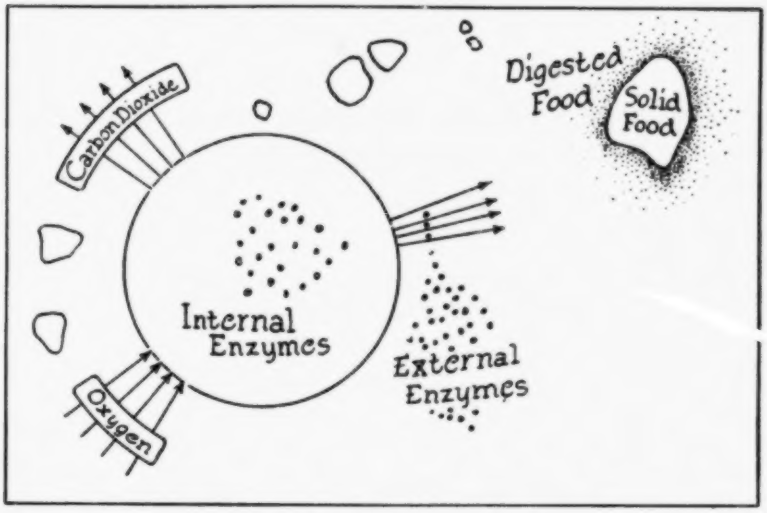


Figure 1

maintaining desirable living conditions for a complicated society of organisms. The efficiency of the filter is not readily explainable on the basis of its simple structure. It is merely a bed of crushed stone or other medium over which the wastes are caused to flow in small streams. It is a method for tremendously increasing the surface area to which the wastes are exposed. The time of passage is about 15 or 20 minutes. Yet biochemical oxygen demand removal is in the neighborhood of 75 or 80 percent, equal to removal by storage in a bottle with excess oxygen for 7 days. This efficiency can be explained only on the basis of biological activity occurring in the filter. The mechanism is not truly that of a filter and yet a definite separation of materials from sewage has resulted from passage through it. The material removed has somehow been collected upon the filter medium.

Whenever water carried organic wastes are allowed to touch, trickle, or flow over a suitable contact surface, slimy films of growth are formed. These gelatinous or zoogleal films make up the normal biological growth on trickling filters and may vary in color from gray to green or red. The growth is widespread over and at different depths and may be variable in nature at different points. It consists of a jellylike matrix in which millions of bacteria are embedded and on which a variety of other organisms find a suitable place to live.

Some of these organisms are in motion swimming in the surface film of moisture, gliding over the filter growth, on the surface of the rock, and in the interspaces. Their life is a continuous scramble to obtain food, to grow, and to propagate. Their interaction in carrying out these requirements results in waste purification.

Certain factors are essential to the maintenance of trickling filter efficiency.

FOOD

The microorganisms have their supply of food pumped to them at all times. It is only necessary for them to take it as it goes by. The quantity, therefore, is adequate. As far as type is concerned, there are carbohydrates, proteins, and some fats plus intermediate decomposition products. They may be in the form of suspended particles, colloidal, or in solution. The living mass has ability to absorb organic matter from solution, coagulate the colloidal matter, and flocculate the finely divided suspended matter. These substances, then, are permanently removed from the fluid or their passage is effectively delayed.

There is a direct relationship between total mass of bacteria produced and the supply of food available. The greater the supply of food, the higher will be the population value. Starting with a small bacterial population, the rate of bacterial increase will follow a sigmoid curve. The leveling off point in the curve will be determined by total food supply. There is, then, equilibrium between bacterial mass and concentration of food substance. If the population ceiling is reached, bacteria are practically inactive in waste purification. Only rapidly multiplying bacteria are efficient in this process. It would appear, then, to be an advantage to keep bacteria always at the maximum growth rate. This can be done only by supplying unlimited food or by preventing the population from reaching the ceiling value. Any method of killing off some, but not all, of the bacteria would do the job. It will be shown, however, that there are natural forces that bring about this result.

BACTERIAL POPULATION

While some trickling filter efficiency can be obtained by the presence of only a single strain of bacteria, a filter contains under natural conditions a wide variety of kinds. The number of kinds will vary with each filter, its condition, the type of waste applied, and the season of the year. It has been found that any increase in the kinds of bacteria present results in an increase in the percent removal of biochemical oxygen demand. This is significant when considered in the light of the kinds and location of bacteria actually found in normally operating filters. The kinds located at different levels have different habitat preferences, utilize different kinds of foods, and produce different end products. The products formed by those in the upper layer make up the food requirements of those in the next layer and so on to the bottom of the filter. There is thus a series of reactions starting with food applied at the top of the filter and ending with the mineral products that pass out in the effluent.

As the wastes pass over the zoogleal mass in the rock filter, soluble substances pass into the matrix, enzymes are added to the fluid, and colloidal and suspended particles adhere to the film and are made soluble. Foods digested by the external enzymes cannot be utilized until they are taken into the bacterial cell where they may serve one of two functions:

1. They may serve as raw material in the repair and growth of the filter life. Usually digestive products of proteins are used for this purpose and are transformed to living matter, there being no immediate products.

2. Food also serves as a source of energy. This is mainly true of carbohydrates and fats. They will be used up in this order of preference (carbohydrates, fats, proteins). The greatest amount of energy is required by bacteria while at the maximum growth rate. Since the release of energy is an oxidation process, biochemical oxygen demand reduction is greatest at this stage also. In the presence of internal enzymes of the proper kind, oxygen combines with the absorbed foods, energy is released, and acids, carbon dioxide, and water, or other products are formed. These pass into the matrix, to the wastes, and into the effluent.

Proteins also are finally used as a source of energy. This type of food is extremely complicated in structure and requires considerable preparation before it is ready for bacterial use. This preliminary process is a digestive one and takes place outside the bacterial cell. Protein is changed to progressively more simple compounds such as proteoses, then peptones, and finally amino acids. Most amino acids are readily soluble and may pass through bacterial membranes and into the cell. Here a fraction of the amino acid unit may be oxidized with the release of free ammonia, hydrogen sulfide, foul smelling mercaptans, and allied substances. They, in turn, may provide energy by being oxidized to sulphates, nitrites, and nitrates. When this stage has been reached, protein has been disposed of and placed on the mineral side of the ledger. Another increment of biochemical oxygen demand has been satisfied.

The organic food is continually changed in character as it passes downward through the filter. The environmental conditions also change continually. It would not be surprising, then, to find bacteria with particular food habits located at particular levels taking up positions where the altered materials reaching them are of the proper kind. It has been found that bacteria in the upper layer are ones capable of decomposing carbohydrates with the production of acids. There are others here capable of digesting proteins and liberating hydrogen sulfide and ammonia from some of the amino acids. In the middle layer bacteria are present which can act upon these products and decompose them further. In the lower layer are forms capable of oxidizing ammonia and nitrites to nitrates, and sulfides to sulfates.

OXYGEN

Biochemical activity in the trickling filter is primarily aerobic. Outside oxygen must, therefore, be supplied for each increment of biochemical oxygen demand satisfied. It is known that the oxygen reserve in the wastes is entirely insufficient. In properly designed filters, natural ventilation is induced by the difference in temperature between the wastes and air. During warm weather an air current moves downward through the filter; in cold weather the air current moves upward. Because of the large filter surface area exposed to the air, oxygen dissolves in the surface moisture, passes through the zoogleal matrix and is available for absorption by the bacterial cells. Sometimes, and for various reasons, a portion of the filter may become clogged. Pooling then results. Oxygen supply will become inadequate in those areas and anaerobic conditions may become established. Filter efficiency will be impaired in proportion to the extent of pooling.

TEMPERATURE

Within limits, temperature has an effect upon the rate of biological activity. As a general rule it may be said that the rate of biological activity approximately doubles for each increase of 10°C. While this statement is generally true, temperature still has only a little effect upon trickling filter efficiency. Rate of activity here apparently is governed by the speed at which organic matter is transferred into the biological film and by seasonal changes in filter population.

BIOLOGICAL ASSOCIATION

So far filter function has been discussed only in the light of bacterial activity. Actually the living matter on the filter is a complex society of a variety of competing organisms. The relative components of the society are not static. Filter growth is a seasonal succession in which certain organisms appear, become prominent, and are in turn replaced by an increasing population of another type. A list of different kinds of organisms in the filter is long and impressive. How long it actually is, no one knows because all the forms have not been identified. But among them both plants and animals are represented. In the plant groups are such forms as bacteria, algae, and molds.

Algae are simple green plants that may occur as single cells or filaments. They depend for life upon their ability to synthesize sugar from carbon dioxide and water in the presence of light. For this reason they occur only in the upper illuminated zone. While they may utilize certain protein digestion products, their purifying role appears to be a minor one. Their primary contribution is probably the addition of oxygen to the wastes that flow by.

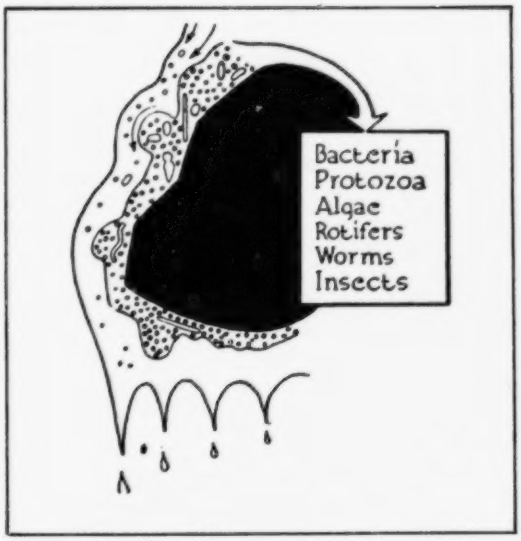


Figure 2

Molds resemble algae in general structure but lack green pigment. They are unable to manufacture sugar and, therefore, must depend upon the food supply in the waste. See Figure 2. While they function in reducing biochemical oxygen demand and in concentrating dissolved matter, they may also function as binders in holding filter growth in place. They are most numerous during winter.

The animal constituents consist of protozoa and metazoa. Protozoa are the simplest of animals. Each consists of a single unit of mass and structure called a cell. It actually is only a blob of jelly-like material held in shape by an elastic membrane. It is capable of all activities necessary to life. Protozoa are classified according to the mechanism by which they move about.

1. Sarcodina -- flowing
2. Flagellata -- whip
3. Ciliata -- cilia

Although flagellates may be present in tremendous numbers, they are small in size, absorb only soluble materials, and have little to do with purification. Many of the Sarcodina and Ciliata are bacterium eaters or predators. The Sarcodina take in bacteria or other food by flowing over and engulfing it. The Ciliata are equipped with an opening or mouth lined with cilia. Currents of water are set up by the cilia so as to sweep bacteria and other particles into the mouth. These are then digested and oxidized for energy and for use as a building material. To this extent biochemical oxygen demand is satisfied and suspended matter is precipitated in the protozoan body.

The more important function of protozoa is their bacterial scavenging action. By attacking and devouring bacteria in the filter there eventually is established a balance between the number of bacterial predators, and a ratio between bacterial population and posible population ceiling. When bacterial population balance is maintained near the zone of maximum growth, biochemical oxygen demand reduction is greater than at the bacterial population ceiling value. The protozoa function then in continually decreasing the bacterial numbers so that bacteria are stimulated to increase their numbers.

Protozoa are concentrated in the upper layer of the filter with the highest number at the two-foot level. This is also about the level of bacterial concentration.

Metazoa are large animals composed of a number of organized cells. In this group are such diverse forms as eel worms, sludge worms, rotifers, water fleas, insect larvae, and certain adult insects. These forms move about through the filter, feeding upon solid material and even on filter growth. Some feed upon the protozoa. Others may burrow through the filter , feeding on all types of organic matter and loosening masses of filter growth. This results in a certain amount of unloading at all times although intensive unloading occurs in fall and spring.

It is apparent, therefore, that the trickling filter is merely a mechanism for maintaining ideal biological conditions. See Figure 3. High rates of biochemical oxygen demand removal result through oxidation of organic compounds and through flocculation and storage. Filter treatment is not completed until unloaded filter growth has been settled and disposed of.

ACTIVATED SLUDGE

The transformation of waste constituents in the activated sludge process is similar in many respects to that of the trickling filter. The biological working units or flocs are kept in suspension and are swept through the waste. They are thus kept continually in contact with their food supply. Large flocs are formed by biological populations and accumulation of waste substances. They function in absorbing materials from suspension and solution and in oxidizing these materials. The results, therefore, are clarification and biochemical oxygen demand reduction. An extreme acceleration of the oxidizing reaction is inherent in this process due to design and development of ideal conditions for growth of desirable biological forms.

It has been determined that the activated sludge floc may vary somewhat in character but always contains a predominance of zoogleal bacteria. Findings by all workers point to the primary importance of these bacteria as the basic functional units in the floc. They are short, rod-shaped with rounded ends, and grow in colonies that are spherical, evenly lobed or tree-like. The gelatinous martix of the colony is composed of the capsules of the bacterial cells. They also may occur free in the medium and swim about by flagellar activity.

The bacteria of activated sludge resemble those of the trickling filter and can function interchangeably in either situation.

Since food material must pass through the bacterial cell wall before it can be used for energy or built into living material, the bacterial surface available determines the purifying efficiency. It has been calculated that each cubic foot in the aeration chamber contains at least 250 square feet of bacterial surface. If free swimming bacteria and protozoa are added the area may reach 500 square feet.

The purification of the wastes by the activated sludge process takes place in two stages. The first of these stages is called clarification. In it carbonaceous and nitrogenous organic matter in all stages of dispersion is removed from the wastes by coagulation, absorption, and other mechanisms and becomes a part of the floc. Clarification is a rapid stage reaching equilibrium in 30 to 40 minutes of passage.

This stage is followed by a reactivation stage during which the clarification powers are restored to the floc. While this is occurring, oxygen is supplied by aeration of the medium, and is used by bacteria to oxidize the food load of the floc. Enzymes of many kinds are effective in the floc matrix and in the waste itself. Complex foods are made soluble and may pass into bacterial cells where in the presence of internal enzymes and the absorbed oxygen supply they are oxidized and energy is released.

For high operating efficiency, it is important here as in the trickling filter that bacteria be maintained at the maximum growth rate. This may be accomplished in two ways; by removal or wastage of sludge and by predation of protezoa and other bacteria feeding organisms. The presence of a large number of ciliates is an indication that activated sludge is in good operating condition.

The activated sludge process can be considered a biological type of filtering apparatus in which the active life rapidly strains out polluting materials and then slowly breaks them down to a non-polluting condition. Purification of milk wastes cannot be considered complete until settled sludge has been drawn out of this system for disposal.

SLUDGE DIGESTION

Digestion is the term applied to the decomposition

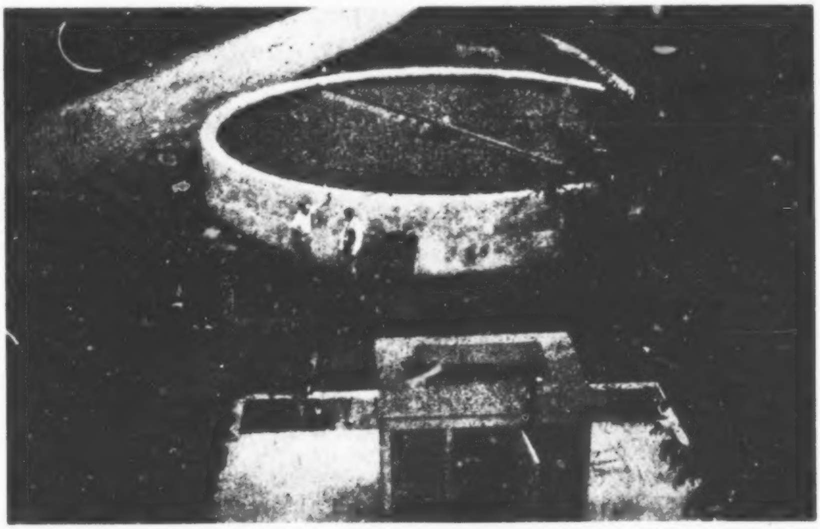


Figure 3 - Trickling filter for treatment of milk plant wastes, Union Center, Wisconsin.

This page is reproduced at the back of the report by a different reproduction method to provide better detail.

of settled sludge. The process is a combination of processes including true digestion by enzymes and reduction and oxidation of compounds by anaerobic organisms. Anaerobic organisms live in the absence of free oxygen and can obtain their supply from oxygen-containing compounds. Digestion is usually carried on in separate sludge digestion tanks. The object of digestion is the alteration of the collected settled solids in order to permit disposal without creating a nuisance. The activities of bacteria, molds, protozoa, and other forms are involved. A wide variety of extra-cellular enzymes are formed by the bacteria and are mixed into sludge. The first stage is characterized by active fermentation involving gas production and formation of organic acids. The gases are mainly methane and carbon dioxide. In the second stage the acids are neutralized or decomposed by alkaline reaction products.

Except for molecular oxygen supply, organisms active in digestion are affected by the same conditions as aerobic organisms. Food must be supplied at frequent intervals by introduction of undigested sludge, temperatures must be maintained at the optimum and the maximum growth rate must be maintained.

These treatment processes embodying combinations of physical, chemical, and biological processes including aerobic and anaerobic methods of milk waste stabilization can prove effective in substantially reducing or eliminating unnecessary steam pollution.

Grateful acknowledgement is extended to Dr. A. F. Bartsch, Senior Biologist of the Wisconsin State Board of Health, for his assistance in the preparation of this paper.

Reproduced With Permission From:
SEWAGE AND INDUSTRIAL WASTES
28(1956): 1219-1231

PROTOZA AND ACTIVATED SLUDGE

Ross E. McKinney and Andrew Gram

Assistant Professor of Sanitary Engineering and Graduate Student, respectively,
Massachusetts Institute of Technology, Cambridge, Mass.

Although protozoa have long been recognized as playing a definite role in the proper functioning of activated sludge, their exact role and interrelationships have never been fully defined. There is little doubt that failure to properly understand the functioning of the various protozoa and bacteria has often resulted in poor design and operation of not only domestic sewage treatment plants but also industrial waste treatment plants. The following study was made to delineate the role of the protozoa and to interpret the results in terms of practical treatment plant operations.

PREVIOUS WORK

The first studies on protozoa in activated sludge consisted of microscopic observations of changes in species as floc developed. Buswell and Long (1), Agersborg and Hatfield (2) and Barritt (3) noted that there was a definite succession of protozoa during the development of activated sludge. During the initial stages of aeration the flagellated protozoa predominated; but with the increased development of floc the flagellates gave way to the free-swimming ciliates. As the activated sludge reached peak efficiency, the stalked ciliated protozoa had come into predominance over the free-swimming ciliates. Other investigators attempted to relate the efficiency of the activated sludge system to the number of protozoa in the mixed liquor. Lackey (4) and Jenkins (5) found that there was little correlation between the protozoan population and the degree of purification. This was further substantiated by the recent work of Baines et al. (6). While numbers were not found to be important, Cramer (7) put forth the concept that protozoa were essential for optimum purification and that routine microscopic examinations for the presence of the protozoa, Vorticella, could be used as an index of a well-operating activated sludge.

Attempts to distinguish the role of the protozoa from that of the other microorganisms in the floc have been made by using toxic chemicals to kill the protozoa and then noting the differences between the treated and untreated samples. Heukelekian (8) and Heukelekian and Gurbaxani (9) used this method to demonstrate that the absence of protozoa resulted in increased effluent B.O.D. and turbidity. While working with pure culture bacterial sludge, Zoogloea ramigera, Butterfield (10) added the protozoan, Colpidium, to one unit and obtained a more efficient system. The most extensive research on protozoa has been conducted by Pillai et al. (11) (12) (13) (14) (15) (16) in India. They advanced the comcept that protozoa were the primary agents in the stabilization of organic matter by activated sludge.

Thus, three concepts of the role of protozoa in activated sludge have been developed by previous investigators:

1. Protozoa are of secondary importance, acting only as polishing agents with regards to effluent B.O.D. and turbidity.

2. Protozoa are of equal importance with bacteria as purifying agents.

3. Protozoa are the primary purifying agents with bacteria acting as secondary agents.

CLASSIFICATION OF PROTOZOA

Protozoa are divided into five classes:

1. Sarcodina
2. Mastigophora
3. Sporozoa
4. Ciliata
5. Suctoria

Of these five classes only three are of any importance in activated sludge: Sarcodina, Mastigophora and Ciliata. The classification of protozoa is based primarily on the method of motility, which is easy to determine microscopically.

The Sarcodina move by means of pseudopodia, commonly called a "false foot." The pseudopodia are formed by shifting protoplasm in the flexible cell membrane. The most common Sarcodina are the Amoeba. The Sarcodina engulf solid food particles, hydrolyze them and absorb them through the cell wall. They also absorb soluble food directly through the cell wall.

The Mastigophora move by means of one or more long flagella. The Mastigophora can be subdivided according to their method of metabolism: holophytic, soluble substrate, and holozoic, primarily solid substrate. The holophytic Mastigophora are further subdivided into the photosynthetic, energy derived from sunlight, and the chemosynthetic, energy derived from metabolism of organic matter. Euglena are photosynthetic holophytic Mastigophora, while Chilomonas are chemosynthetic holophytic Mastigophora. The holozoic protozoa are chemosynthetic and are capable of deriving energy from soluble as well as solid food. Oikomonas are a holozoic Mastigophora.

The Ciliata move by means of many short cilia and utilize organic particulate matter for food. The Ciliata are subdivided into the free-swimming forms and the stalked forms. Paramecia and Tetrahymena are examples of free-swimming ciliates and Vorticella of stalked ciliates.

METABOLISM AND GROWTH

The primary purpose of metabolism is to supply the necessary building blocks and the energy required to produce protoplasm for new cells. The growth of any microorganism, whether protozoa, bacteria, algae, fungi, etc., is dependent upon the metabolic requirements of the organism, the chemical nature of the substrate and the physical environment. Each species of microorganisms has its own definite metabolic requirements. Thus, for a given set of enviromental conditions the growth of a pure culture of a single species of microorganisms in a dilute substrate such as encountered in most liquid organic wastes is controlled by the quantity of metabolite in solution.

The growth of two species of microorganisms both capable of utilizing the same substrate will depend upon their respective rates of metabolism. If both species are the same size and metabolize at the same rate, their growth will be equal. If both species are the same size but one metabolizes faster than the other, the faster metabolizing species will predominate. If the two species are of unequal size but metabolize at the same rate, the smaller species will produce greater numbers but the mass of protoplasm will be equal. Thus it can be seen that the mass of cellular protoplasm is the correct corollary for determining true competitive action rather than numbers of organisms which has been used so extensively in the past.

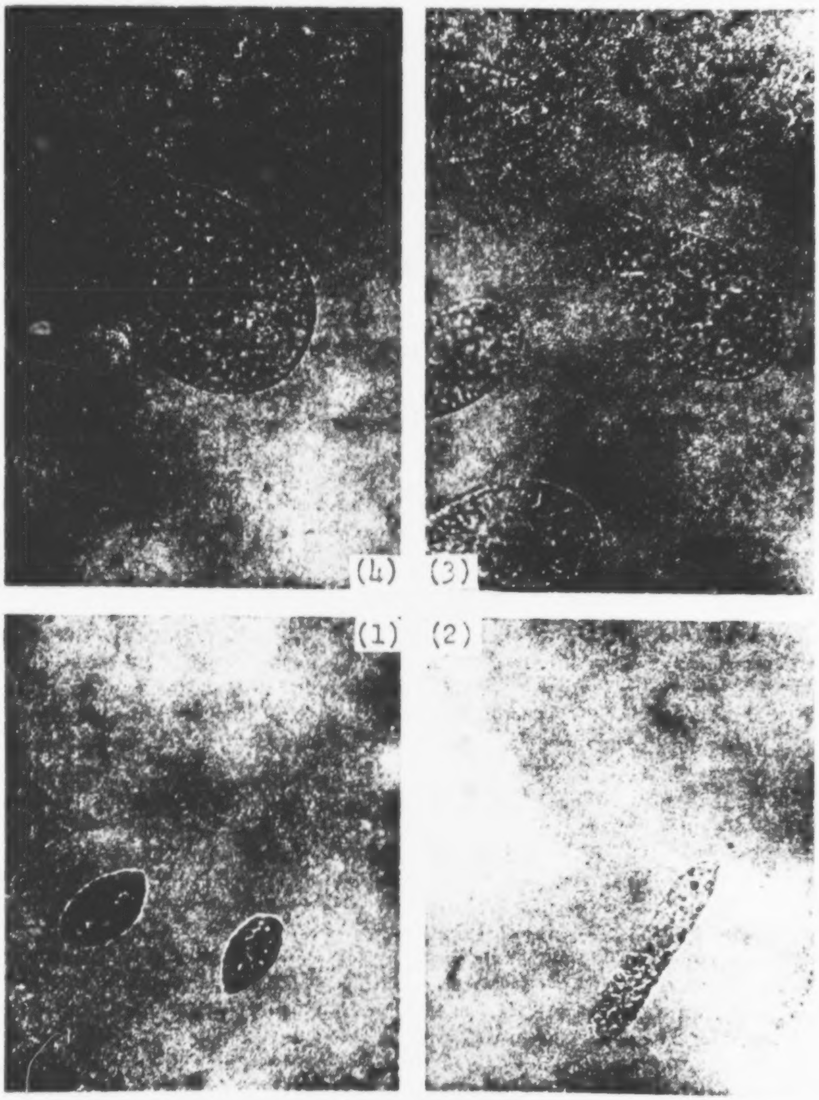
The growth of two species of microorganisms which do not utilize the same components of the substrate is the same as if the two species were being grown separately. The two species are not competing for food and grow independently of each other.

The third case of growth is that of two species of microorganisms, one species predatory to the other. The growth of the prey is dependent upon the substrate while that of the predator is dependent upon the prey. In a completely soluble substrate the prey will grow rapidly, followed by the predator. As the number of predators increases the demand on the prey increases until the prey is extinct. Needless to say, the predator also soon becomes extinct. In an environment where there is a hiding place for the prey such as floc or other particulate matter the predator is slowed down in its search for prey and extinction is prevented.

The basic corollaries of microorganism growth cited previously are discussed in detail by Gause (17). He presents the complete mathematical evaluation of growth, both non-competitive and competitive. From these basic corollaries it is possible to predict the fate of the various microorganisms in any biological waste disposal system. While the ensuing discussion and data will primarily be concerned with protozoa and activated sludge, it should be remembered that the same phenomena are occurring in other types of waste disposal systems and in the stream itself.

Protozoa, bacteria, algae and fungi are the common microorganisms which gain entrance to waste disposal systems primarily from the soil. Their survival depends upon their ability to successfully compete for food. The algae do not predominate primarily because of their need for sunlight. The fungi are affected by many factors and often predominate over the bacteria. Since the bacteria and fungi belong to the same family of microorganisms, it is actually wrong to speak of them as being separate groups; but common usage has necessitated such distinction. Since the bacteria predominate over the fungi under normal environmental conditions, let us consider the competition of protozoa and bacteria with the understanding that the use of the term "bacteria" also includes fungi.

The bacteria are dependent upon soluble food. They have extracellular enzymes which are capable of converting solid organic matter into soluble organic matter. Their size, averaging 1.5 x 1.0 microns, is such that they require very little organic matter to produce a new cell and are able to produce tre-



This page is reproduced at the back of the report by a different reproduction method to provide better detail.

Figure 1 - Photomicrographs of protozoa studied (magnified approximately 3600 times), (1) Chilamonas paramecium, (2) Euglena gracilis (3) Tetrahymena gelii, and (4) Glaucoma scintillans.

mendous numbers on very little food in a very short period of time. One part per million of organic matter is capable of producing 10^6 bacteria per milliliter.

Considering the metabolism of the bacteria and the protozoa, it can be seen that the *Sarcodina* and the chemosynthetic holophytic *Mastigophora* must compete with the bacteria for their food; while the holozoic *Mastigophora* and the *Ciliata* are predators on the bacteria. At the start of an activated sludge system, the food concentration is high and the population of any group of microorganisms is small. As a result all organisms should be able to grow with the *Sarcodina* and the chemosynthetic holophytic *Mastigophora* being the predominate protozoa during the early phases. The small size of the bacteria and their low metabolic requirement permit them to obtain a larger and larger share of the organic matter as their numbers increase. Competition of this type is such that with the continuous reseeding, as is practiced in activated sludge, the bacteria cannot completely eliminate the holophytic protozoa. The numbers of holophytic protozoa are greatly reduced by this competition and they can be considered as removed as far as their practical significance is concerned.

The holozoic protozoa must wait until the bacteria increase to sufficient numbers before they can find enough food to grow. Thus, the number of holozoic protozoa always lags the number of bacteria in relation to their growth phases. The tiny holozoic flagellates grow first because of their lower energy requirements; but the ciliates overgrow them as a result of the ciliates' ability to obtain food more rapidly.

While previous investigations and theoretical concepts tended to affirm the general relationships between the protozoa and the bacteria, it was felt that laboratory experiments were necessary to demonstrate the competition and survival of the holophytic flagellates and the ciliates with bacteria under well defined and controlled conditions. The following laboratory studies were made with that purpose in mind.

EXPERIMENTAL METHOD

In order to determine the relationships which exist between the protozoa and the bacteria making up the floc in activated sludge systems, the experiments were set up under the concept of "minimum conditions." "Minimum conditions" were established as (a) a completely soluble, nutritionally balanced sub-

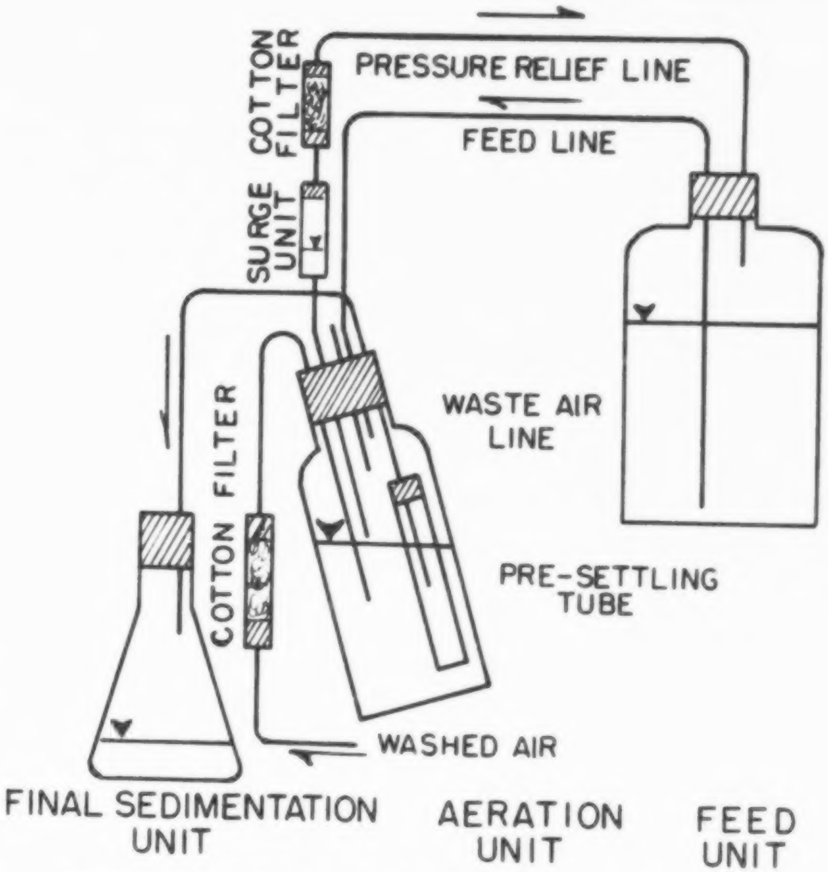


Figure 2 - Schematic diagram of laboratory continuous flow unit.

strate; (b) a mixture of bacteria isolated from activated sludge; and (c) a pure culture of the protozoa being studied. A completely soluble substrate was used to prevent the formation of a "pseudo" floc in the aeration unit resulting from agglomeration of colloidal solids rather than the microorganisms. All floc formed during these experiments was the direct result of metabolic activity of the microorganisms in the mixed liquor. The substrate had the following formula:

Nutrient broth, Difco, 200 p.p.m.
Glucose, 200 p.p.m.
Potassium phosphate, monobasic, 110 p.p.m.
Ammonium sulfate, 100 p.p.m.
Sodium chloride, 30 p.p.m.
Potassium chloride, 7 p.p.m.
Magnesium sulfate, 5 p.p.m.
pH adjusted to 7.2 with N/1 NaOH.

The 5-day B.O.D. was approximately 290 p.p.m.

Recent investigations (18) had shown that under proper environmental conditions most of the bacteria isolated from activated sludge were capable of forming floc. Therefore, no attempt was made to isolate or to use any special floc-forming bacteria in this study. It was necessary to have only protozoa-free bacteria in the aeration system. The bacteria were separated from the protozoa of a laboratory developed activated sludge by streaking an agitated sample of sludge onto nutrient agar plates and picking off representative colonies of the predominating organisms. Since one of the components of the soluble substrate was also the major source of nutrients in nutrient agar, this

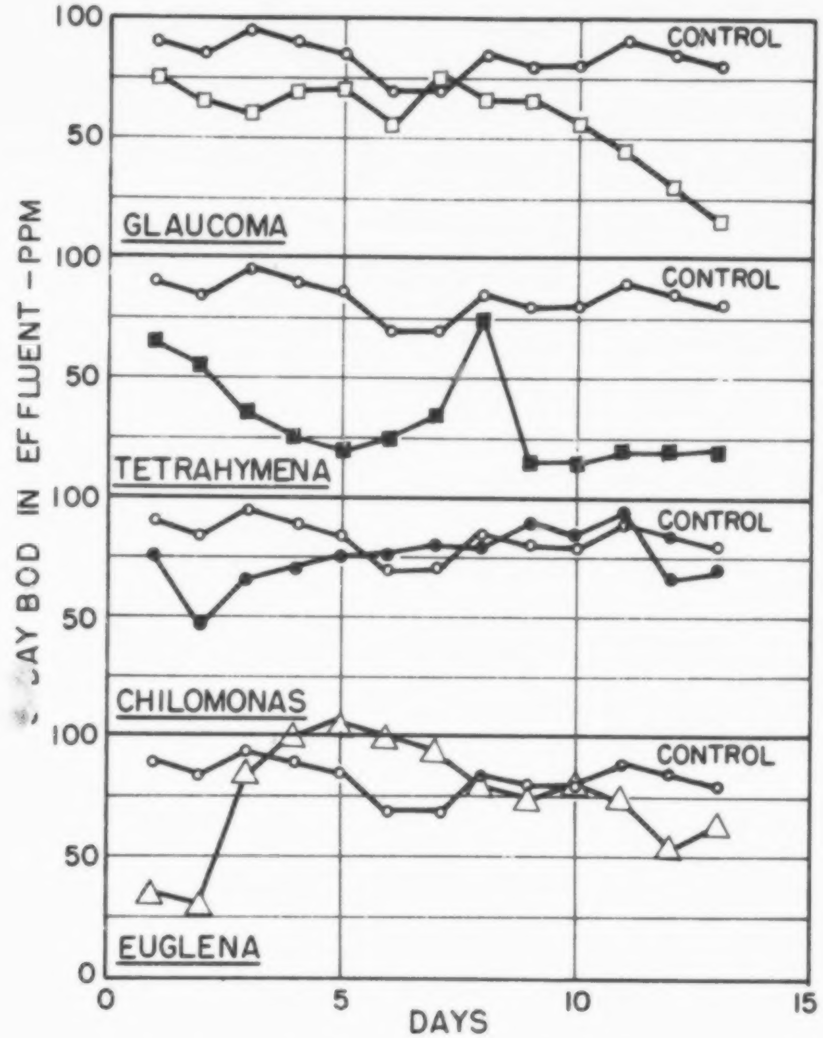


Figure 3 - B.O.D. of effluents from continuous flow units.

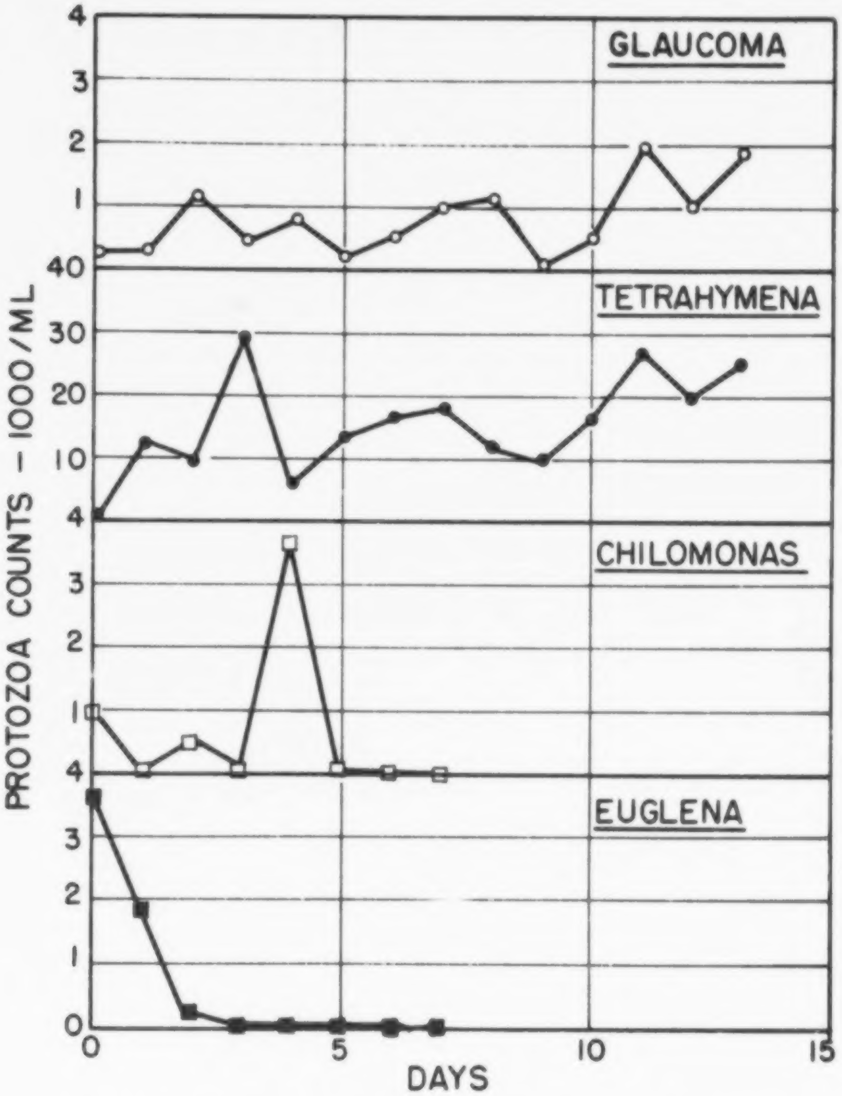


Figure 4 - Protozoa counts in effluents from continuous flow units.

insured the isolation of the major species of aerobic bacteria capable of metabolizing the substrates.

Pure cultures of representative species of flagellate and ciliate protozoa were obtained from the culture collection of G. W. Kidder, Amherst College, Amherst, Mass. These included as flagellates, Chilomonas paramecium and Euglena gracilis, and as ciliates, Tetrahymena gilii and Glaucoma scintillans. Photomicrographs of these organisms are shown in Figure 1.

The activated sludge systems, Figure 2, were continuous flow units consisting of a 4-l. feed reservoir which fed a 500-ml. aeration chamber containing 300-ml. of mixed liquor and a 300-ml. sedimentation unit. The entire system operated by gravity flow with the hydraulic head on the discharge siphon from the aeration tank to the sedimentation unit acting as the rate controller. The size of the systems permitted sterilization of each system as a unit before the start of the run. Each unit was capable of operating 12 days on a single feed reservoir with a detention period of 24 hr. in the aeration tank. After sterilizing and cooling the aeration units, they were opened for bacterial inoculation. The systems were allowed to operate 24 hr. without the addition of fresh substrate to permit the bacteria to reach a high population level. The protozoa were added to the aeration units and continuous feeding was started. Composite samples of the effluents were collected over a 24-hr. period and analyzed for B.O.D. and turbidity. Protozoa counts in the effluent were made from grab samples collected while changing the sed-

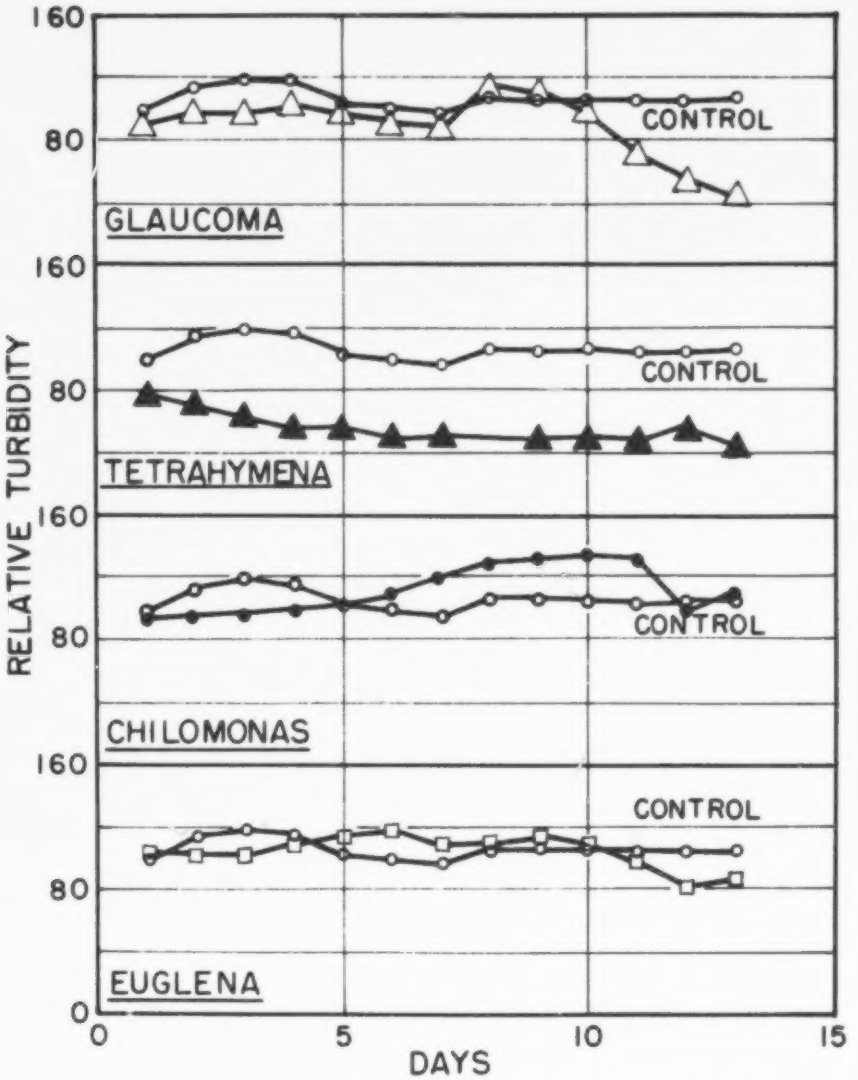


Figure 5 - Relative turbidity in effuents from continuous flow units.

Table I. Aeration system after 12 days of operation

ORGANISM	MIXED LIQUOR SUSP. SOLIDS (p.p.m.)	B.O.D. (p.p.m.)		PROTOZOA (NO./ml.)	
		UNFILTERED EFFLUENT	FILTERED EFFLUENT[1]	EFFLUENT	MIXED LIQUOR.
CONTROL	400	79	23	0	0
TETRAHYMENA	650	20	23	25,300	17,300
GLAUCOMA	580	15	14	1,880	3,360
CHILOMONAS	560	68	22	0	0
EUGLENA	390	65	14	0	0

[1] MICROFILTERED

imentation flasks. After 13 days of operation samples were removed from the aeration units and placed in Warburg flasks containing fresh samples of the substrate. The oxygen utilization characteristics of each sludge were determined from continuous readings on the Warburg Respirometer. Suspended solids determinations and protozoa counts were made on samples of the remaining sludge.

RESULTS

The B.O.D. data of the composite effluent samples are shown in Figure 3. The control sample reached equilibrium very rapidly with only a slow increase in efficiency during the remainder of the run. The units containing the ciliates showed greater B.O.D. removals than the control unit and a definite downward trend as the sludges became more stabilized. The flagellate units showed a sharp dip and then a rise approaching the level of the control. Variations in effluent B.O.D. were found to follow closely the number of protozoa in the effuent as shown in Figure 4. Relative turbidity measurements on the effluent samples were made with a Coleman Nephlometer using 100 p.p.m. of diatomaceous earth as a standard and are shown in Figure 5.

Samples of the mixed liquor were analized at the end of the run and the data are shown in Table I. Membrane * filters were used to determine if the source of the B.O.D. in the effluent was related directly to the organisms or to unused substrate. The membrane filter retained all of the bacteria and the protozoa while permitting the soluble substrate to pass. Analyses of the effluent before and after filtration yielded the amount of B.O.D. directly attributable to the microorganisms.

The oxygen utilization curve shown in Figure 6 followed the general trend of the other data with the sludges containing the ciliates utilizing oxygen more rapidly than the control or units containing the flagellates. These units also contained the largest quantities of sludge. The maximum rates of oxygen utilization of all of the sludges were approximately the same, 65 mg. of oxygen per hour per gram of sludge. The rates of endogenous metabolism of the sludges were approximately 10 mg. of oxygen per hour per gram of sludge as they were removed from the aeration units and averaged 4 mg. of oxygen per hour per gram of sludge over a 24-hr. period.

DISCUSSION

The laboratory data showed primarily that it was possible to demonstrate the general principles of survival and competition of protozoa with bacteria in activated sludge for two major groups of protozoa, holophytic flagellates and ciliates. In addition to affirming the work of previous investigators, it helps to tie together certain field observations with the theoretical concepts, a technique much needed in the field of sanitary engineering where theory is often regarded as having no relationship whatsoever to field practice.

The principles of bacterial flocculation and stabilization as set forth by Butterfield (10) were once again demonstrated in the control unit. The various pure culture bacteria in the control unit utilized the soluble organic matter, produced new cells and underwent flocculation in a manner similar to normal activated sludge. But unlike normal activated sludge, the pure bacterial floc did not clarify the effluent completely upon quiescent settling. The motile,

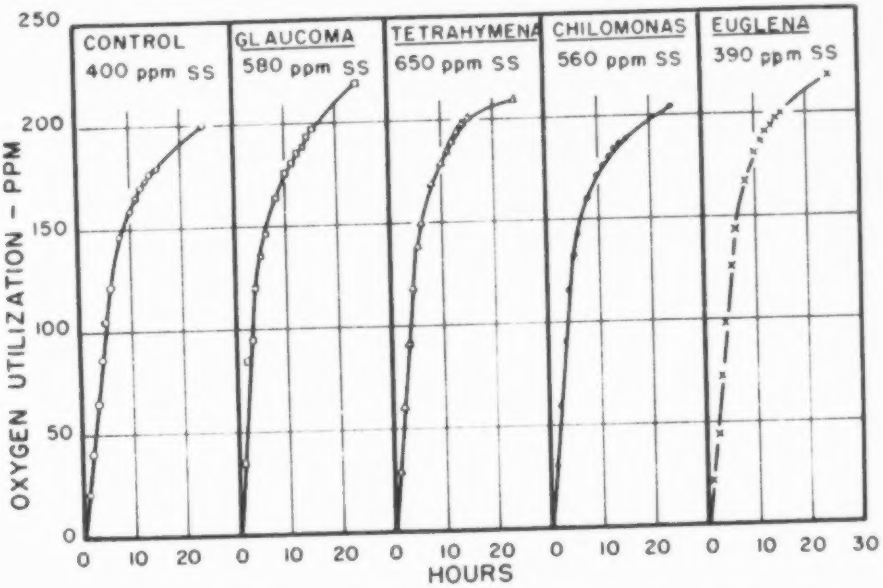


Figure 6 - Oxygen uptake by activated sludges from continuous flow units.

---

*"Millipore" brand membrane filter.

actively metabolizing bacteria remained in the supernatant after the floc had settled. These motile bacteria were found to be responsible for the major portion of the effluent B.O.D.

Adding the holophytic flagellates to the pure bacterial floc permitted evaluation between two different biological groups which had similar metabolism. The flagellates were not able to successfully compete for the food and rapidly died off. The larger size of the flagellate gave less surface area to absorb food than the bacteria and at the same time created a greater demand for food per unit organism. The low initial food concentration did not help the flagellates even though they were able to grow in the substrate in the absence of the bacteria. Combining these observations with previous investigations it can be seen that the presence of flagellates in activated sludge systems will be brief at most. Their appearance will be made when the bacteria population is low and the food concentration high. As soon as the bacteria increase in numbers the flagellates are unable to compete and become insignificant in the activated sludge.

On the other hand the ciliates grew quite satisfactorily in the presence of the pure bacterial floc. The free swimming ciliates fed on the bacteria, and increased in numbers throughout the test run. The ciliates lived to a large extent on the free-swimming bacteria, reducing the effluent turbidity and B.O.D. to levels encountered in normal activated sludge systems. The presence of floc appeared to retard the ciliates in their search for food and, thereby, to limit the rate of growth. The resultant equilibrium between the bacteria and the ciliates was mutually favorable. Together they produced more floc than the bacteria alone, a clearer effluent, a more stable effluent and a more rapid rate of oxygen uptake. Thus, it becomes obvious that the ciliates play a very important role in the proper operation of activated sludge.

The fact that the ciliates were able to grow in the substrate in the absence of bacteria but not sufficiently to give a satisfactory oxygen uptake in Warburg studies adds additional information contrary to the concept that the protozoa are the primary purifying agents in activated sludge. In the system studied, as in most, if not all activated sludge systems, the bacteria were the source of food for the ciliates, not the original organic substrate. Actually, the bacteria utilize the substrate and are the primary agents of stabilization. The protozoa act as population stimulators by removing the excess bacteria which are formed, permitting a lower bacteria-substrate equilibrium and a more active floc to result.

Naturally, the question arises as to how many ciliates are required to produce a good activated sludge. There is no answer to that question since it depends upon the system itself. This has been clearly pointed out by previous investigators who have tried to correlate protozoa numbers to efficiency and have found no correlation to exist. Numbers of protozoa in a system are meaningless because it is impossible to tell whether the microorganism are increasing or decreasing. As a given activated sludge system becomes more efficient the numbers of any type of protozoa rise, reach a peak and then decline. Two samples taken at two definitely different operating efficiencies can yield the same number of a given species of protozoa. Thus, it is obvious that protozoa counts in themselves cannot be used as an index of efficiency.

PRACTICAL SIGNIFICANCE

More important to the treatment plant operator is the type of protozoa present. As indicated pre-

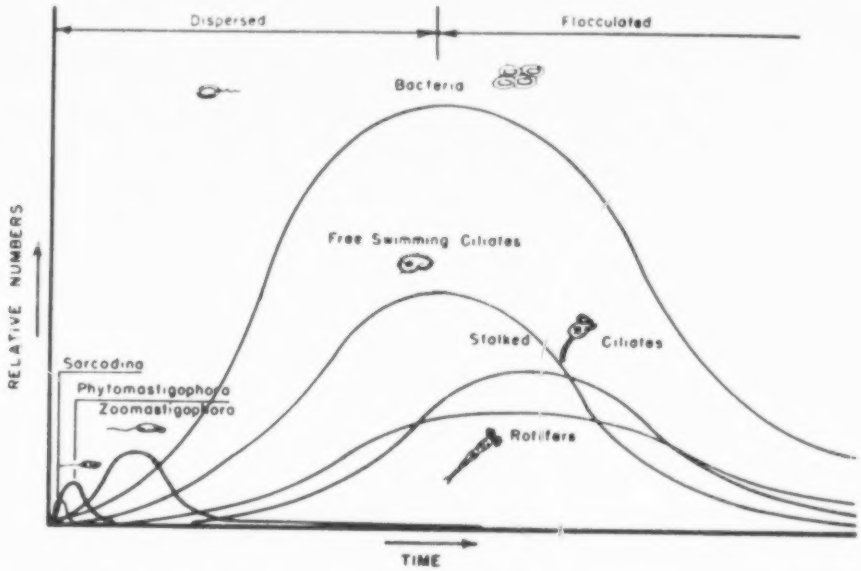


Figure 7 - Relative predominance of microorganisms in activated sludge systems.

viously, there is a definite succession of protozoa as activated sludge is formed and becomes more efficient. Knowledge of the various protozoa and their significance can be used as a valuable guide to better activated sludge operation. Observations of the protozoa can be made quickly with a microscope of only 100 power. These observations reflect the immediate condition of the sludge. Industrial waste systems fed unusual or toxic organic compounds require careful control to insure continuous operation at maximum efficiency. Since the protozoa are more sensitive to toxic compounds than bacteria, observations on the change in protozoa from normal levels give an immediate indication of trouble before it has had time to affect the bacteria.

Each operator must determine the protozoa characteristics of his own system and relate their activities to efficiency. A general guide as to relative predomination of protozoa and efficiency in an activated sludge system can be stated as follows and is shown in Figure 7.

1. Sarcodina predominate very rarely and only in systems just starting or just recovering from complete toxicity.

2. The holophytic Mastigophora (flagellates) predominate at low efficiency when the organic concentration is high. In some industrial wastes systems where the organic load is high, the efficiency may be mathematically high but the indication is that the effluent still contains a high concentration of organic matter.

3. The holozoic Mastigophora (flagellates) arise as the nolophytic flagellates decrease. They indicate an only slightly more efficient system and efforts to distinguish between the two types of flagellates is not warranted by the results.

4. The free-swimming Ciliata (ciliates) are found when there are a large number of free-swimming bacteria. The efficiency of the system when the numbers are very high is approximately 50 per cent. They can indicate a fairly efficient system or a poor one. The key is in the other types present. Flagellates and free-swimming ciliates are at the low side of the efficiency scale while the presence of stalked ciliates and higher animal forms such as rotifers indicates the high side of the scale.

5. The presence of stalked ciliates indicates an activated sludge with a low B.O.D. effluent. The stalked ciliates arise as a result of the number of available bacteria being reduced below the demands of the free-swimming ciliates. Having lower energy requirements than the free-swimming ciliates the stalked ciliates survive. A very stable activated sludge system will have very few stalked ciliates and, usually, no other protozoa forms. Actually, the ciliates are measuring the bacteria activity; but since the bacteria activity reflects the biochemical condition of the system, the ciliates reflect the efficiency with considerable accuracy.

The major problem is that the operator and the engineer fail to realize that the biological indices of operation reflect the complete and instantaneous condition of the system. Microscopic observations are simple and easy to make. The protozoa characteristics are obvious to even the least trained operator. Confidence in the significance of protozoa observations on a routine basis is difficult to obtain but, once obtained, it is never lost.

SUMMARY

The work of previous investigators was correlated with the theoretical concepts of competition and predomination between species by laboratory demonstrations. The succession of protozoa as observed in the formation of activated sludge was discussed from the aspect of operational control. The significance of the protozoa to the optimum operation of activated sludge was emphasized.

REFERENCES

1. Buswell, A. M., and Long, H. L., "Microbiology and Theory of Activated Sludge." Jour. Amer. Water Works Assn., 10, 309 (1923).

2. Agersborg, H. P. K., and Hatfield, W. D., "The Biology of a Sewage Treatment Plant--A Preliminary Survey--Decatur, Illinois." Sewage Works Jour., 1, 4, 411 (July, 1929).

3. Barritt, N. W., "The Ecology of Activated Sludge in Relation to its Properties and the Isolation of a Specific Soluble Substance from the Purified Effluent." Ann. App. Biol., 27, 151 (1940).

4. Lackey, J. B., "Biology of Sewage Treatment." Sewage Works Jour., 21, 4, 659 (July, 1949).

5. Jenkins S. H., "Role of Protozoa in the Activated Sludge Process." Nature, 150, 607 (1942).

6. Baines, S., Hawkes, H. A., Hewitt, C. H., and Jenkins, S. H., "Protozoa as Indicators in Activated Sludge Treatment." THIS JOURNAL, 25, 9, 1023 (Sept., 1953).

7. Cramer, Robert, "The Role of Protozoa in Activated Sludge." Ind. Eng. Chem., 23, 309 (1931).

8. Heukelekian, H., "Partial and Complete Sterilization of Activated Sludge and the Effect on Purification." THIS JOURNAL, 3, 3, 369 (July, 1931).

9. Heukelekian, H., and Gurbaxani, M., "Effect of Certain Physical and Chemical Agents on the Bacteria and Protozoa of Activated Sludge." Sewage Works Jour., 21, 5, 811 (Sept., 1949).

10. Butterfield, C. T., "Studies of Sewage Purification. II. A Zoogloea-forming Bacterium Isolated from Activated Sludge." Pub. Health Rpts. 50, 671 (1935).

11. Pillai, S. C., "The Function of Protozoa in the Activated Sludge Process." Current Sci., 10, 84 (1941).

12. Pillai, S. C., and Subrahmanyan, V., "Role of Protozoa in the Activated Sludge Process." Nature, 150, 525 (1942).

13. Pillai, S. C., and Subrahmanyan, V., "Relation of Protozoa to Bulking of Activated Sludge." Sci. and Culture, 8, 376 (1943).

14. Pillai, S. C., and Subrahmanyan, V., "Role of Protozoa in the Aerobic Purification of Sewage." Nature, 154, 179 (1944).

15. Pillai, S. C., Wadhwani, T. K., Gurbaxani, M. J., and Subrahmanyan, V., "Relative Efficiency of Bacteria and Protozoa in the Flocculation and Oxidation of Organic Matter Suspended in Water." Current Sci., 16, 340 (1947).

16. Pillai, S. C., Wadhwani, T. K., and Subrahmanyan, V., "Respiration of Vorticella." Current Sci., 16, 341 (1947).

17. Gause, G. F., "The Struggle for Existance." William & Wilkins Company, Baltimore, Md. (1934).

18. McKinney, R. E., and Weichlein, R. G., "Isolation of Floc-Producing Bacteria from Activated Sludge." Appl. Microb., 1, 259 (1953).

Reproduced With Permission From:
LIMNOLOGY AND OCEANOGRAPHY
2(1957): 77-84

BIOLOGICAL FACTORS IN TREATMENT OF RAW SEWAGE IN ARTIFICIAL PONDS

A. F. Bartsch and M. O. Allum[1]

Robert A. Taft Sanitary Engineering Center, U. S. Public Health
Service, Cincinnati, Ohio and Department of Zoology,
South Dakota State College, Brookings, South Dakota

ABSTRACT

Ninety-nine communities in the northern plains area use artificial ponds as the sole method of sewage treatment. Five ponds, selected for variability in design and loading, were studied intensively during each season of one year.

A typical biota principally composed of algae and bacteria becomes established and participates in the treatment process. Reduction in coliform density (most probable number) was 99% more than half the time and always 95% or greater. Oxygen demand (B.O.D.) was typically reduced by 70-90%. Total suspended matter was reduced 80% or more in winter, but sometimes was increased up to 258% in summer as result of algal production.

Various factors affect the treatment process. Depth of the euphotic zone ranged from 0.1-0.7 meter. Bottle tests gave oxygen production rates between zero and 3.6 $g/m^2/hr$ at different times of day, and total respiration rates from 0.1-2.4 $g/m^2/hr$. Oxygen demand from contributed sewage was only 6-7% of total respiratory demand. Vertical gradients, diurnal variations, ice cover, and other factors prevent simple projection of bottle test data to the ponds.

INTRODUCTION

In the northern plains area draining to the Missouri River 99 communities use artificial ponds as the sole method of sewage treatment. These communities, which represent a total population of about 125,000 persons, are distributed by States as follows:

Colorado	9	Nebraska	3
Kansas	1	North Dakota	38
Minnesota	2	South Dakota	19
Missouri	7	Wyoming	5
Montana	15		
		Total	99

Depending upon community population these ponds range in size from 1/7 acre to 130 acres. Because of their low cost they have become extremely attractive to the small municipality in which per capita cost of conventional treatment is considerably greater than in larger municipalities. New sewage ponds can be built for about $4.50 to $20.00 per capita, or about 1/8 to 1/2 the cost of a conventional plant. In keeping

[1] At time of the survey reported here, M.O. Allum was on staff of the South Dakota State Health Department.

with present design practice, artificial ponds provide one acre four feet deep for each 100 persons of connected population.

Reduced to simplest terms the stabilization mechanism in a sewage pond is mutually beneficial interaction between bacteria, algae, and various other plankters, which eliminates the objectionable characteristics of sewage. Digestive and oxidative activities of bacteria stabilize organic matter and simultaneously produce CO_2, ammonia, and other nutrients needed by the algae. The latter, through photosynthesis, liberate excess oxygen which helps to maintain a desirable aerobic system. In the overall process relatively unstable organic matter is converted principally to a more stable algal mass. These interrelationships between algae and bacteria have been studied intensively in California by means of pilot plant facilities that have a much shorter detention period than ponds used in the northern plains area (Ludwig and Oswald 1952, Oswald and Gotaas 1955).

Growth in popularity of raw sewage ponds in the northern plains area since 1950, and evident interest in using them shown by municipalities in other regions, emphasize the urgent need for better information on how they function, how they should be constructed, and how their efficiency might be improved. Accordingly, a seasonal study of five ponds was made during the period January 1955 to June 1956 as a cooperative undertaking of the Public Health Service and the State Health Departments of North and South Dakota. This field survey is the initial phase of a continuing comprehensive study of pond treatment of wastes. The objectives were to evaluate structural, seasonal, and loading variations and to point up areas of approach and direction to be taken in pilot plant studies. The material presented in this paper is based entirely upon observations made during the initial field study.

Of five raw sewage ponds studied during each season of 1955, two have been selected for presentation because they represent the lowest and the highest B.O.D. loadings encountered. The pond at Lemmon, South Dakota, with an area of 25 acres serves 2,600 persons at a mean B.O.D. loading of 6.84 pounds/acre/day. At Kakoka, South Dakota, the raw sewage pond has an area of 3.0 acres and serves 800 persons at a mean B.O.D. loading of 22.9 pounds/acre/day.

Various factors, such as loading, solar radiation, depth, wind, temperature, and ice cover, influence the course and velocity of the stabilization process. Although these factors have profound effects upon various physiological reactions, and some of these are partially known, impact upon photosynthesis is of greatest immediate interest and importance.

DATA

The results of pond treatment are impresive. B.O.D. concentrations typically are reduced in a seasonal range of 70-98%. Reductions in coliform bacterial densities, in terms of M.P.N., can be expected to exceed 95% at all times and 99% more than half the time. Total suspended matter was reduced 80% or more in winter, but sometimes was increased up to 258% in summer as a result of algal production. Freedom from odor nuisances, scarcity of floating solids, and the green natural appearance do not suggest a sewage origin of the pond content.

Continuing emphasis is placed upon photosynthesis in aquatic communities in relation to primary production of organic matter (Steeman-Nielsen 1937, Ryther 1954, 1956a, 1956b, Verduin 195[illegible], 1956). As a rule in such studies oxygen production is mainly significant as a step toward the measurement of primary production. In contrast to this, photosynthetic oxygen evolved in raw sewage ponds is a more important consideration than the organic matter produced concurrently in the process. Effectiveness of oxygen production has a practical value in relation to sewage loading and optimum depth. Although oxygen production in relation to light and depth is considered here solely from this point of view, considerable attention has been directed by others to cultivation and harvesting of algae grown in sewage (Gotaas, Oswald and Ludwig 1954).

Three distinct considerations are involved in the importance of solar radiation and help determine pond efficiency and the area and depth needed. First, regional variations in annual solar radiation, which are governed by latitude, elevation, and cloud cover, will influence pond design in a given location. Second, seasonal changes in daily solar radiation, which may vary locally by a factor of three or more, suggest the seasonal difficulties to be expected. Finally, penetration of incident light determines how much of the pond volume will participate in oxygen production.

Penetration of light in raw sewage ponds is strikingly less than in most bodies of water for which data are available (Clarke 1939, Birge and Juday 1929-32, Whitney 1938). Figure 1 shows light penetration in three South Dakota bodies of water, as measured by matched surface and submerged photronic cells (Weston, Model 856 RR) sensitive in a range from 420-700 mμ. The transparency of Murdo Lake, which is a water supply reservoir, is not unusual with an extinction coefficient of 0.58 per meter. In this lake, the rate of light absorption increased with depth. The euphotic zone, the lower boundary of which by definition is located at 1% of surface light, extended to a depth of 7.16 meters. In the sewage stabilization pond at Kadoka the corresponding depth was only about 0.4 meter! There is little doubt that dense algal populations in this and other highly fertile raw sewage ponds are responsible for the rapid light extinction observed. Systematics and growth features of algae in somewhat similar sewage oxidation ponds in California have been studied intensely by Silva and Papenfuss (1953) and Allen (1955).

Because of these interesting light relations and the current practice of constructing raw sewage ponds with an average depth of 4 feet (1.22 meters), it seemed desirable to estimate photosynthetic oxygen production as a step in evaluating this benefit to treatment from each increment of excavated depth.

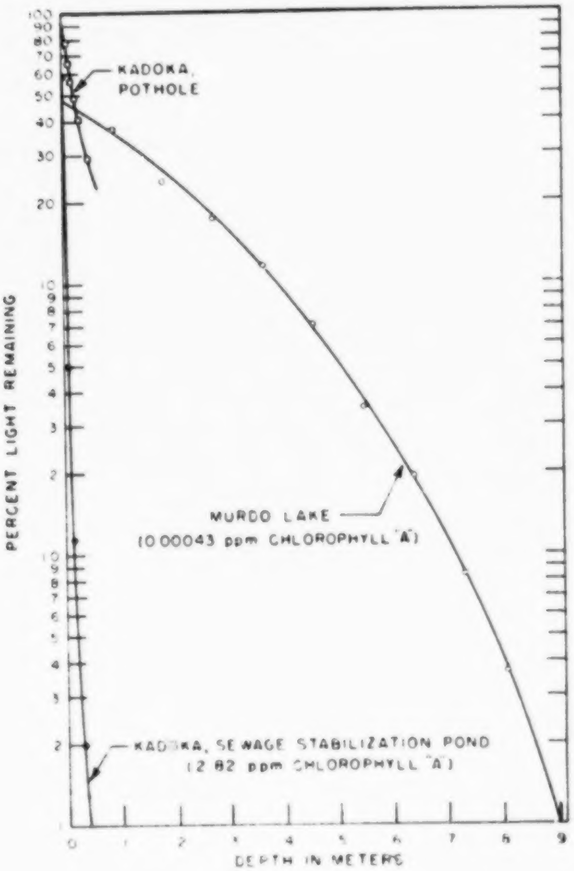


Figure 1 - Light remaining at various depths in three different bodies of water, July 27-29, 1955.

it seemed desirable to estimate photosynthetic oxygen production as a step in evaluating this benefit to treatment from each increment of excavated depth. A sample of sewage pond content taken from a depth of 20 cm was distributed into 2 series of 300-ml glass-stoppered bottles, which were suspended at selected depths in chicken-wire baskets. Half the bottles at each depth (usually duplicates or triplicates) were covered with two layers of aluminum foil to exclude light. At termination of the exposure period dissolved oxygen concentrations were determined using the Alsterberg (Azide) modification of the Winkler test (APHA, AWWA, and FSIWA 1955) for comparison with initial values similarly obtained.

In one test of five-hours' duration at the Lemmon Pond (Table 1), bottles were hung at four depths giving a range in mean light intensity from 3.8 to 1050 foot-candles. Oxygen production at 25.4 cm was seven times as great as at 60.9 cm, and 29 times as great as at 96.5 cm. At 83.0 cm, at which 1% of surface light remained, oxygen production was estimated to be only 0.2 ppm/hr. If the production rate between 25.4 cm and the surface is taken arbitrarily as 2.9 ppm/hr, the production in the euphotic zone was then 1.2 g/m^2/hr (or 9.2 pounds/acre/hr), based upon a mean production of 1.4 ppm. Simultaneous respiratory demand in the euphotic zone was 0.3 g/m^2/hr, or only 25% of the total production. This relationship is not actually as favorable as it appears, as will be shown in subsequent discussion.

TABLE 1. *Oxygen production at different depths 10:00 AM–3:00 PM, August 12, 1955, at Lemmon, South Dakota*

Determinations were made in triplicate

% surface light	Approx. ft. candles	Depth cm	D.O. change, ppm		Oxygen production ppm/hr	Respiration ppm/hr
			Light bottles	Dark bottles		
19.0	1050	25.4	12.6	−2.1	2.9	0.4
3.1	167	60.9	0.0	−2.1	0.4	0.4
0.5	27	96.5	−1.4	−2.0	0.1	0.4
0.07	3.8	134.6	−1.6	−1.8	0.04	0.4

Verduin (1956) has found that the ratio of mean photosynthesis in the euphotic zone to the rate under optimal light conditions is 0.65. If that ratio applies to the present test conditions, and if 2.9 ppm/hr is assumed to approximate the rate under optimal light, the resulting mean production rate in the euphotic zone would be 1.9 ppm/hr. This compares favorably with the estimated weighted average of 1.4 ppm/hr determined from measurements at several depths. Also, using Verduin's listed yield of 0.22 micromoles of oxygen evolved per microgram of chlorophyll per hour, the calculated sewage pond yield under optimal light, with 0.46 ppm of chlorophyll, would be 3.2 ppm/hr. These, of course, are optimal rates of production which do not prevail throughout the day as shown in tables that follow.

Raw sewage ponds differ from other standing bodies of water in the intensity of photosynthesis and respiration in the shallow surface layer and the rapidity with which light decreases with depth. At no time was a pond found with a euphotic zone deeper than 0.7 meter, and one as shallow as 0.1 meter was measured. Difficulties in suspending light and dark bottles at precise light levels and in determining exact depth of optimal light are apparent.

Tests similar to that described above were done during four different daylight periods in ponds at Lemmon and at Kadoka. At Lemmon (Table 2), where the euphotic zone was then about 83 cm thick, bottles were hung at 25.4, 60.9, and 96.5 cm to give 19.0, 3.1, and 0.5% of incident light intensity, respectively. A separate pond sample was used for each test. Variations resulting from this procedure are suggested by differences in the respiratory values.

Oxygen production varied from 2.1 g/m^2/hr at

TABLE 2. *Relation of photosynthesis to respiration at different times of day, August 10, 1955, at Lemmon, South Dakota*

Time of day	Duration of measurement, hours	Light intensity ft. cand.		Dissolved oxygen relations gm/m²/hr			
		25.4 cm	9.65 cm	Total photosynthetic production	Total respiratory use	Surplus	Observed sewage influent B.O.D. loading (ultimate)
9:00 AM	0.5	552	5.5	2.1	0.9	1.2	0.04
1:00 PM	1.0	780	7.8	1.5	0.7	0.8	0.04
3:00 PM	2.0	390	3.9	0.8	0.7	0.1	0.04
7:30 PM	2.0	55	<1.5	0.03	0.6	−0.6	0.04

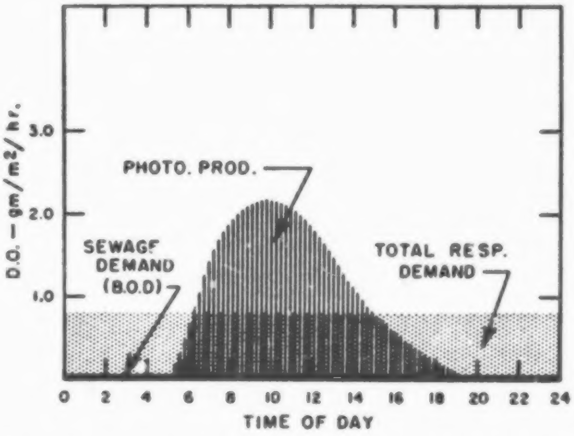


Figure 2 - Oxygen relations in a typical raw sewage pond.

midmorning to 0.03 g/m²/hr during early evening. Cause for the lower production at 1:00 P.M. than at 9:00 A.M. is not definitely known but very likely resulted from depletion of available CO_2. However, if total respiratory use largely reflected phytoplankton density at the depth of sampling, oxygen production per algal quantity during the two periods was not greatly different.

To evaluate the photosynthetic system in relation to the sewage load for which oxygen must be furnished, daily ultimate B.O.D. load was simply converted to grams of oxygen demand/m²/hr. While admittedly this is not a precise procedure because sewage flow fluctuates widely and the oxygen demand of the sewage is not identifiable in the complex demand of the pond system, it is sufficiently accurate to permit gross comparison of oxygen demand from sewage with that of total respiration. At first glance it seems highly inefficient to create a biological system with an oxygen use rate of 0.7 g/m²/hr in order to obtain the 0.04 g/m²/hr needed to stabilize the sewage. This relationship is shown graphically in Figure 2. Cheap land, low construction costs, and limited maintenance requirements make this apparent inefficiency of less consequence than would otherwise be the case.

Similar bottle tests were conducted in the raw sewage pond at Kadoka. As shown in Table 3 sewage loading was twice that observed at Lemmon, and both oxygen production and total respiration were more intense in response to the heavier loading.

Although bottle-test data have been treated as if they apply directly to the ponds, there are many enviromental differences between them that must be reconciled. Some of these are related to a procession of diurnal changes. Pond temperature, for example, follows changes in air temperature in a cyclic fashion. Temperature gradients, as shown in Figure 3, generally are established on calm days and disappear readily during windy weather and gradually after sunset as do those classified in Pichler's "Small ponds" category (Young and Zimmerman 1956).

Vertical distribution of phytoplankton as indicated by chlorophyll concentration also follows a diurnal pattern in which concentration near the surface at mid-day may be strikingly greater than a few centimeters deeper. This pattern is suggested by the data in Table 4, and is further supported by chlorophyll determinations on samples of pond overflow collected at three-hour intervals over a 24-hour period. Table 5 gives the range of chlorophyll concentrations found in the bodies of water samples. As one would expect, chlorophyll concentration increases with increased loading.

Dissolved oxygen concentrations are more transient in the ponds than in the test bottles. During a 24-hour period dissolved oxygen concentrations at a given depth were observed to vary from zero to 35 ppm or more in following a daily repetition of midday highs and nocturnal lows. At one extreme oxygen bubbles escape from the pond surface, at the other anaerobic conditions prevail. At mid-day, vertical variations have been found to range from 30.3 ppm at 25.4 cm to zero at 91.4 cm. As shown in Figure 4, the vertical and diurnal patterns result from different rates of gain and loss at each depth. Because the euphotic zone extended to only 40.6 cm at this time, oxygen gain below 60cm after mid-day was most probably the result of diffusion from the highly productive overlying layer.

Mainly because of seasonal changes in phytoplankton density, light absorption and thickness of the euphotic zone change with season and even from day to day. Light absorption characteristics during spring, summer, and fall are shown for the Lemmon pond in Figure 5, and for the Kadoka pond in Figure 6. Deviations from a straight-line plot are caused by uneven vertical distribution of

Table 3. *Relation of photosynthesis to respiration at different times of day, July 28, 1955, at Kadoka, South Dakota*

Time of day	Duration of measurement, hours	Light intensity ft. cand.		Dissolved oxygen relations gm/m²/hr			
		7.6 cm	43.2 cm	Total photosynthetic production	Total respiratory use	Surplus	Observed sewage influent B.O.D. Loading (ultimate)
9:30 AM	0.5	945	2.7	3.6	1.9	1.7	0.09
2:00 PM	0.5	2830	8.1	2.3	2.4	~0	0.09
4:30 PM	0.5	750	2.1	2.4	1.2	1.2	0.09
8:30 PM	0.5	<1.5	<1.5	0.0	0.1	~0	0.09

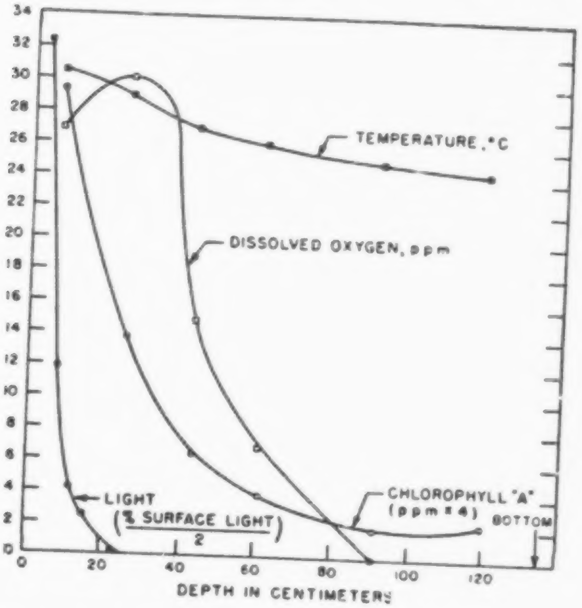


Figure 3 - Vertical variations in water quality characteristics, 12:15-12:30 P.M. July 28, 1955, Kadoka, South Dakota. Values of the ordinate apply to temperature and dissolved oxygen directly and to chlorophyll "a" and light as indicated.

phytoplankton as described. Limits of the euphotic zone at Lemmon were more variable than at Kadoka.

In all ponds studied transparency was less during spring and fall than during summer. This is shown in Table 6, which also gives the limits of the euphotic zone and the extinction coefficient for each body of water.

There is generally no measurable oxygen production during winter under ice, and consequently anaerobic conditions are continuous from the onset of ice cover to ice breakup. It is not possible to discern clearly each causative factor from the others, but light extinction alone would drastically limit photosynthesis even if other factors were favorable.

Table 4. *Chlorophyll "a" concentrations (in ppm) at various depths at different times of day, July 27, 1955, Kadoka, South Dakota*

Depth in cm	10:45 AM	12:30 PM	5:45 PM
7.6	—	7.32	3.28
25.4	—	3.45	—
30.5	3.36	—	—
43.2	—	1.62	2.62
60.9	2.86	0.99	1.53
91.4	2.22	0.49	0.92
119.4	—	0.38	0.53

Table 5. *Chlorophyll "a" Concentrations (in ppm) July 29–August 20, 1955*

	7.6 cm depth	121.9 cm depth
Kadoka, South Dakota	2.82	0.35
Wall, South Dakota	0.18	—
Lemmon, South Dakota	0.47	0.44
Maddock, North Dakota	0.08	0.06
Wishek, North Dakota	0.65	1.05
Murdo Lake, South Dakota	0.0004	0.0002

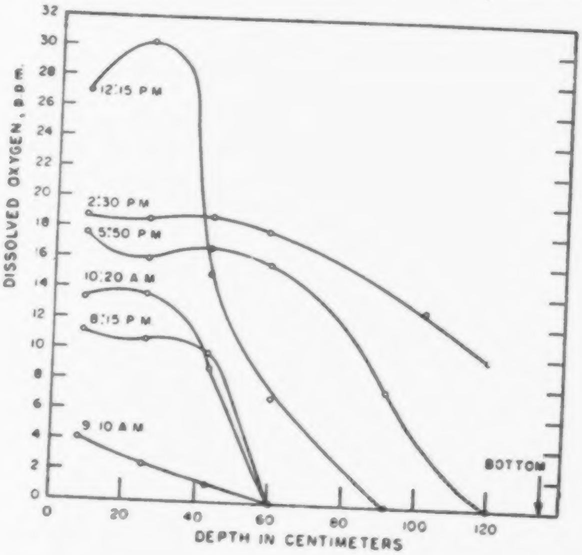


Figure 4 - Vertical variations in dissolved oxygen at different times of day, July 28, 1955, Kadoka, South Dakota.

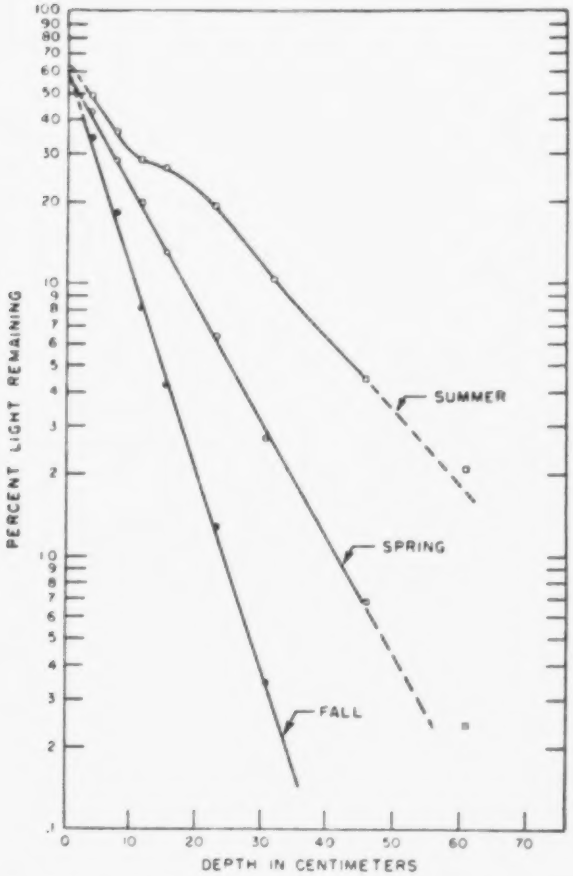


Figure 5 - Light remaining at various depths during spring, summer, and fall, 1955, Lemmon, South Dakota.

Depending upon thickness and variation in clarity, light-transmission through ice and snow ranged from 55.5% to 1.2% (Table 7). These figures agree closely with measurements by Greenbank (1945) on several southeastern Michigan lakes. He noted that "cloudiness" of the ice caused by minute air bubbles or dirt had great influence upon light transmission. The contrast in per cent transmission between Lemmon and Kadoka similarly is the result of clear ice at the former and opalescent ice at the latter. In these observations ice quality appeared more important than thickness, and quality generally increased as B.O.D. loading decreased.

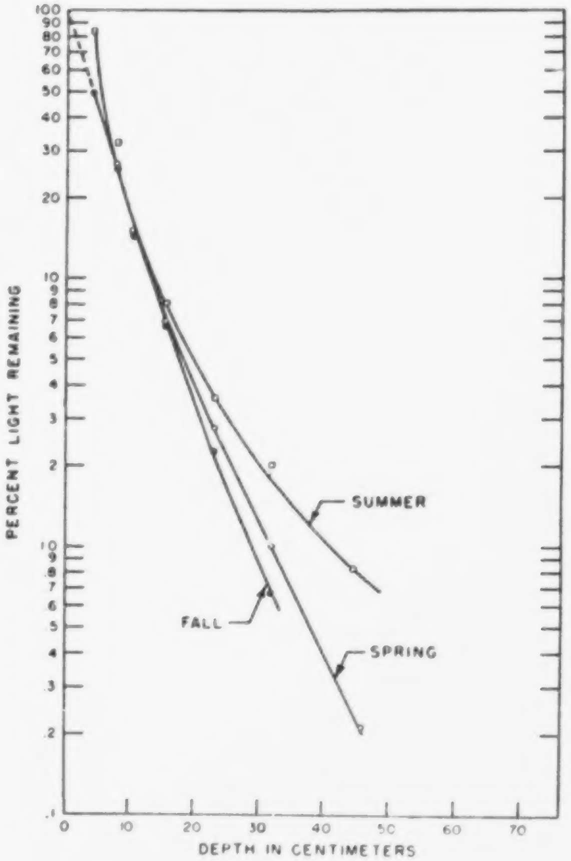


Figure 6 - Light remaining at various depths during spring, summer, and fall, 1955, Kadoka, South Dakota.

SUMMARY

Dissolved oxygen relations are the key to freedom from nuisance conditions and acceptibility of raw sewage ponds as municipal treatment facilities. Light and dark bottle tests offer one means of exploring oxygen relations as affected by controllable factors such as loading and depth. Estimated oxygen production, using the factors given by Verduin (1956), agrees fairly well with the test data . Ryther's (1956a) expression for determining absolute photosynthesis in the ocean, and pond design equations that have been developed (Gotaas, Oswald, and Golueke 1954) are other approaches to be explored in raw sewage ponds. Vertical variations in pond content, diurnal and seasonal effects, weather changes and other factors, emphasize the restraint with which such data may presently be applied.

TABLE 6. *Approximate depth in cm at which indicated light remains*

	10% of Surface Light	1% of Surface Light	Extinction Coefficient*
Kadoka, South Dakota			
Spring	12.7	31.8	14.8
Summer	14.0	40.6	9.6
Fall	12.7	29.2	16.0
Wall, South Dakota			
Spring	5.1	10.2	46.0
Summer	17.8	41.9	10.7
Fall	5.1	15.2	22.9
Lemmon, South Dakota			
Spring	17.8	41.9	9.9
Summer	33.0	69.9	5.9
Fall	10.2	24.1	17.4
Maddock, North Dakota			
Spring	14.0	30.5	13.7
Summer	25.4	58.4	7.0
Fall	19.1	39.4	10.8
Wishek, North Dakota			
Spring	7.6	17.8	22.4
Summer	16.5	39.4	10.8
Fall	8.9	21.6	18.4
Kadoka, South Dakota, Pothole			
Summer	—	—	2.55
Murdo Lake, South Dakota			
Summer	4,013	7,162	0.58

* Extinction coefficient = k in equation $I/I_0 = e^{-kl}$ with l expressed in meters.

REFERENCES

Allen, M. B. 1955. General features of algal growth in sewage oxidation ponds. Publ. No. 13, Cal. State Water Poll. Contr. Bd., Sacramento, pp . 1-47.

APHA, AWWA, and FSIWA .1955. Standard methods for examination of water, sewage and industrial wastes. 10th Ed. New York, Amer. Publ. Health Assoc., pp. 255-256.

Birge, E. A., and C. Juday. 1929-32. Solar radiation and inland lakes. Trans. Wis. Acad. Sci. Arts Lett., 24-27.

Clarke, G. L. 1939. The utilization of solar energy by aquatic organisms. Problems in Lake Biology; A.A.A.S. Publ. No. 10, pp. 27-38.

Gotaas, H.B., W.J. Oswald, and H. F. Ludwig. 1954. Photosynthetic reclamation of organic wastes. Sci. Mon., N.Y., 79: 368-378.

Gotaas, H.B., W. J. Oswald, and C. Golueke. 1954. Algal-bacterial symbiosis in sewage oxidation ponds. Fifth Prog. Rept. Berkeley, Cal., San. Eng. Res. Lab., Univ. Cal., pp. 1-88.

Greenbank, J. 1945. Limnological conditions in ice-covered lakes, especially as related to winter-kill of fish. Ecol. Monogr., 15: 343-392.

Ludwig, H. R., and Oswald, W. J. 1952. Role of Algae in Sewage Oxidation Ponds. Sci. Mon., N.Y., 74: 3-5.

TABLE 7. *Light transmission through ice and snow, January 22–February 10, 1955*

Sewage stabilization pond	Date	Mean ice thickness cm	Mean snow thickness cm	Mean surface light ft. cand.	Mean light transmission per cent
Kadoka, South Dakota	Jan. 22	26.7	2.3	1086	6.5
Wall, South Dakota	Jan. 25	25.6	0.0	608	20.4
Lemmon, South Dakota	Jan. 31	32.3	0.0	1337	55.5
Maddock, North Dakota*					
Cell 1	Feb. 5	66.0	7.6	1300	1.2
Cell 2	Feb. 5	66.0	7.6	1460	5.1
Cell 3	Feb. 5	62.2	5.1	1100	3.5
Wishek, North Dakota	Feb. 10	43.2	7.6	1490	1.2

* Installation consists of three ponds or cells connected in series.

Oswald, W. J., and Gotaas, H. B. 1955. Photosynthesis in sewage treatment. Proc. Amer. Soc. Civil Eng., 81, Separate No. 686, pp. 1-27.

Ryther, J. H. 1954. The ratio of photosynthesis to respiration in marine plankton algae and its effect upon the measurement of productivity. Deep-Sea Res., 2: 134-139.

Ryther, J. H. 1956a. Photosynthesis in the ocean as a function of light intensity. Limnol. & Oceanogr., 1: 61-70.

Ryther, J. H. 1956b. The measurement of primary production. Limnol. & Oceanogr., 1:72-84.

Silva, P. S., and G. F. Papenfuss. 1953. Systematic study of the algae of sewage oxidation ponds. Publ. No. 7, Sacramento, Cal., State Water Poll. Contr. Ed., 1-35.

Steeman-Nielsen, E.S. 1937. The annual amount of organic matter produced by the phytoplankton in the sound off Helsingor. Medd. Komm. f. Danmarks Fiskeri-og Havundersogelaer. Serie: Plankton, Bd. III, No. 3, 38 pp.

Verduin, J. 1951. Photosynthesis in naturally reared aquatic communities. Plant Physiol., 26: 45-49.

Verduin, J. 1956. Primary production in lakes. Limnol. & Oceanogr., 1: 85-91.

Whitney, L. V. 1938. Transmission of solar energy and the scattering produced by suspensoids in lake waters. Trans. Wis. Acad. Sci. Arts Lett., 31: 201-221.

Young, F. H., and J. R. Zimmerman. 1956. Variations in temperature in small aquatic situations. Ecology, 37: 609-611.

Reproduced With Permission From:
ECOLOGY
40(1959): 273-291

TRICKLING FILTER ECOLOGY

Wm. Bridge Cooke

Robert A. Taft Sanitary Engineering Center, U. S. Department of Health
Education, and Welfare, Public Health Service, Cincinnati 26, Ohio

INTRODUCTION

According to Clarke (1954), "the fundamental steps in the operation of an ecosystem are: (1) reception of energy; (2) production of organic material by producers; (3) consumption of this material by consumers and its further elaboration; (4) decomposition to inorganic compounds; and (5) transformation to forms suitable for the nutrition of the producers. If the area is occupied by a self-sufficient community, all these steps will go on within it."

On a large scale, and on the basis of a large population of microscopic or near-microscopic organisms, a trickling filter embodies many of the characteristics of the independent ecosystem as outlined above. However, the trickling filter is a man-made ecosystem and few man-made ecosystems are truly self sufficient. The trickling filter is built for one purpose, the treatment of sewage by the action of a biota upon it. This biota is independent only as long as sewage is applied to the filter. The development of such a biota is relatively rapid. Its members are derived from the population of surrounding soils, waters, or habitats in which decaying or decomposable materials are present, as well as from sewage.

Basically the trickling filter population is composed of decomposers, transformers, and locally, of producers which are partly dependent on the other members of the biota.

HABITAT

In the United States today there are three principal methods of sewage disposal. One of the commonest is the direct release of polluted water into the nearest stream. A refinement on this process is found in the primary treatment of sewage in which the sewage is directed into settling basins with or without the addition of chemicals. After settling out of solid materials, the liquid is added to that of the nearest stream. However, purification (removal, by natural or artificial methods, of objectionable matter from water) usually results in only 25%-40% BOD removal and approximately up to 70% suspended solids removal. The biochemical oxygen demand

(BOD) of sewage is the quantity of dissolved oxygen, in mg/ul, required during stabilization of the decomposable organic matter by aerobic biochemical action (Standard Methods, 1955). A further refinement is effected by any one of several biological treatment processes, commonly through the use of activated sludge or trickling filters. A sanitary engineer whose duties include maintenance of a sewage treatment plant thus becomes a biological engineer using tools of applied ecology.

A number of terms to be used in the description of trickling filters may be defined according to the Glossary of Water and Sewage Control Engineering (1949) for a clearer understanding of some of the problems to be discussed. In the following discussion, quoted terms and definitions are from this source. The broadest definition of sewage is "the water supply of a community after it has been fouled by various uses." This may be broken down into the two most important types of pollution, domestic or sanitary sewage and industrial wastes. The latter may be processed in special treatment plants, or in conjunction with domestic sewage in municipal plants of the primary or secondary type. The municipal treatment plant operator usually is concerned with the former although he realizes that some industrial wastes are included.

The operation of a secondary sewage treatment plant is based on one or another method of "biochemical-," "biological-," or "sewage oxidation," a . . . "process whereby, through the agency of living organisms in the presence of oxygen, the organic matter contained in sewage is converted into a more stable or a mineral form." Among the principal techniques of exposing sewage to the oxidative actions of microorganisms are the activated sludge process and the trickling filter. The latter is the only method with which we are concerned in this report. The anaerobic digestion of sludges is not considered here.

In the literature of sewage purification a number of terms have been used to describe the trickling fileter. This structure is defined as follows: "A filter consisting of an artificial bed of coarse material, such as broken stone, clinkers, slate, slats or brush, over which sewage is distributed and applied in drops, films, or spray, from troughs, drippers, moving distributors, or fixed nozzles, and through which it trickles to the underdrains, giving opportunity for the formation of zoogleal slimes which clarify and oxidize the sewage." The process is termed "biological filtration." The structures have been termed "bacteria beds," "contact beds," and "percolating filters." The modification in which the spray comes from fixed nozzles has been termed a "sprinkling filter." Depending on the rate sewage is applied to the filter after primary settling, the filter may be termed "high-rate" (dosed at 10-30 million gallons per acre per day-mgad), or "standard-rate" or "low-rate" (dosed at 1-4 mgad). Since the Orford Subcommittee (1957) prefers the use of "low-rate" to "standard-rate" this term is used in the following discussion. Other modifications of the trickling filter are in use but the work on which this report is partly based has been done on low-rate and high-rate trickling filters.

As indicated above, the trickling filter exists to support a biological community referred to as a "zoogleal slime." This has been referred to commonly as a "microbiological film" which is defined as "the gelatinous film of zooglian growths covering the media or spanning the interstices of a biological bed." "Zoogloea," in the terminology of sewage treatment, is defined as a "jelly-like matrix developed by bacteria. The word is usually associated with activated sludge growths in biological beds." The bacteriologist is more specific in his use of this term. To him Zoogloea is a distinct genus of bacteria, as well as the type of bacterial growth produced during certain phases of the life cycle of single, colonial, or filamentous bacteria which can produce gelatinous matrices.

Finally, the term "degree of purification" may be mentioned. This phrase is defined as "a measure of the removal, oxidation, or destruction of solids, organic matter, bacteria or other specified substance effected by sewage treatment processes." The degree of purification of sewage represents the efficiency of the sewage treatment plant. This rating can be used as a means of obtaining more funds with which to build a more efficient plant, or as a means of evaluating the residual waste products of a municipality which are being emptied into streams. The purity of such water is important to other communities downstream and to the biota of the stream.

The trickling filters studied most intensively by the writer are those in the Dayton, Ohio, sewage treatment plant. This plant is primarily concerned with the treatment of domestic sewage. The conditions of the influent are relatively constant since only rarely do loads of industrial wastes exceed the capacity of the plant to handle them with the domestic sewage. Eight of the 20 filters are the high-rate type and are dosed continuously throughout the year. The remaining 12 filters are of the low-rate type and receive the excess portion of the settled sewage. Their dosage is, therefore, essentially, intermittent, occurring only during periods of the day when the plant is receiving peak loads. Living conditions for the biota of the low-rate filters are different from those of the high-rate filters. Data on effectivity of operation of the two types are not available since effluents are composited before analysis.

The filters are 165 feet in diameter, 7.5 feet deep, constructed in the form of a concrete cylinder with a funnel-shaped channeled base, and are filled with more than 160,270 cubic feet of limestone slag. On the porous surface of this rock a population of microorganisms has developed. Because it is impractical to dig deeply into this mass of rock, which varies in size between two and five inches in diameter, only the surface layer of stone has been examined periodically and regularly.

Since the habitat is man-made, and since it was made for a specific purpose, it is reasonable to expect that conditions in the habitat will be modified from time to time to increase the efficiency of the structure and its population. The most noticeable effects on the filter biota are produced by alteration of dosing rates, when the filters are shut down for repairs or when excessive run-off from precipitation dilutes the sewage.

Effects of changeable air temperature on the filter surfaces are ameliorated by the relatively constant temperatures of the continuous dosage of settled sewage.

Relatively little ponding occurs in the filters. Spaces between the stones are large enough in most cases so that cross growth has not occurred and the filters are well ventilated. Conditions of excessive septicity rarely, if ever, develop.

Biotic effects of the population appear to be relatively constant. If disseminules of additional species reach the site and the species becomes effective in the population, little change will result since the new species will have replaced a species unable to maintain the competitive level. However, it is not to be implied that numbers of species are fixed. Effects of various groups of species in the population are relatively constant since they are apparently controlled by interaction and available food supplies. A possible exception could be found in seasonal over-population of snails or worms neither of whose influence is reflected in the activities recorded in the weights of material found on sampling slides or in species or colonies of organisms isolated therefrom.

POPULATIONS

Introduction

The biotic populations of the trickling filter are relatively uniform in composition. That is, bacteria, fungi, protozoans, algae, nematodes, rotifers, snails, sludge worms, and larvae of certain insects of which the foregoing are composed, may vary from filter to filter, at the species level, but the biota, whether the filter is in the United States or in Europe, remains relatively constant. Occasionally, when conditions permit, other organisms may also be found such as the mosses reported at Dayton (Cooke 1953). Such organisms are accidental invaders, are restricted to marginal areas, and rarely complete their life cycle, remaining in the vegetative state.

In the past identification of the members of this population has been of little concern to the sanitary engineer as long as the population became established and functional relatively quickly after placing a filter in operation. A good operating condition is one in which little clogging occurs in the spaces between stones and one which carries on its function of removing solid, colloidal, or dissolved materials from the sewage, thus reducing its biochemical oxygen demand.

The presence of bacteria has been considered normal. The species which are present have not been evaluated beyond the requirement that the coliform content of final sewage discharged into the receiving stream be low or lacking. Fungi have been considered nuisances more than aids in the biological treatment of sewage (Becker 1954; Cooke 1954). Tomlinson in England (1941, 1946) has found that several species of fungi are members of the population, as have Hesseltine (1953) and Cooke (1957). On the other hand, Becker and Shaw (1955) report that the occurrence of fungi is seasonal and of little significance. Lackey has surveyed the protozoan populations (1924) in New Jersey while intensive population studies have been carried on with these organisms in England. The occurrence of algae has been noted occasionally according to a survey of Stanbridge (1955). His finding seems odd since algae are invariably present on the surface of trickling filter rock in the many filters which have been observed by the writer. Insect populations have been studied in England relatively intensively (Hawkes 1952), because certain species have become nuisance problems in that country discovery of control methods has appeared to be important. Recently problems of snail control have been considered by Ingram et al. (1958) in connection with equipment damage and nuisance problems.

Composition of Microbial Film

To date, few comprehensive studies have been published in which the biota of the zoogleal slime has been described. Bacteriologist, mycologists, algologists, entomologists, or protozoologists have been interested in one filter or another, at the surface or more rarely at several depth levels, and have reported on their observations without overlapping into the interrelated fields. Occasionally a few organisms from one or more of the other members of a population have come to the attention of the observer but the resulting lists are incomplete. Consequently, a composite list of organisms is presented to indicate the complexity of the populations on the trickling filter. It would be difficult for one individual to study the whole population and its interrelationships comprehensively since the organisms involved cover such a wide range of groups of species in plant and animal kingdoms.

A report by Heukelekian and Crosby (1956) includes relatively representative chemical and physical data on the composition of zoogleal slimes. Since it is difficult to get materials by direct scrapings from trickling filter stone, unglazed tiles were exposed in channels carrying sewage to obtain slime growths of similar composition to those found on trickling filter stones. Table I indicates the type of physical and chemical data of interest to the sewage treatment plant operator.

TABLE I. Analysis of slimes (after Heukelekian and Crosby, 1956)

Determination	Results
pH	8.6
Density at 20° C	1.0122
Moisture, % of wet weight	95.4
Total solids, % of wet weight	4.6
Volatile solids, % of total solids	70.7
Ash, % of total solids	29.3
Total nitrogen, % of total solids	1.0*
Ammonia nitrogen, % of total solids	0.5
Grease, p.p.m.	15.7

*This determination is low for a mass of organisms but materials on which the determination was based include sludges and other inert matter which would tend to reduce the ratio of nitrogen to other elements present.

In studying the populations of the sewage treatment plant and its included processes, the bacteriologist is interested primarily in the occurrence of coliform bacteria. This group has long comprised one of the most important groups of organisms indicative of sewage pollution (Kabler 1957). High coliform density indicates a high degree of pollution. One requirement of effective sewage purification in some communities is the removal of as many coliform bacteria as possible. Thus, beyond routine checks of m.p.n. (most probable number) of coliform bacteria, whether done by the Standard Methods (1955) or the membrane filter (Clark *et al*. 1951) tehnique, little attention is paid at present to other kinds of bacteria present on trickling filters. No comprehensive species lists have been found in the literature for this habitat. However, Hotchkiss (1924) prepared a list of bacteria by their activities on sprinkling filter beds in New Jersey. An average of 20-24 samples obtained throughout the year gave the following numbers of bacteria in thousands per cc. of filter slime; nitrate reducers 208.4, H_2S producers (from protein) 172.9, albumen digesters 102.6, species which oxidize nitrites to nitrates 53.3, species which oxidize NH_4 salts to nitrates 39.8, H S producers (from sulfates) 19.1, and species which reduce thiosulfate to sulfate 2.0.

As early as 1914, Johnson, in England, observed bacteria and fungi on filters. He considered that *Sphaerotilus natans* was among the important bacteria on the beds he studied and that it took the form of *Zoogloea ramigera* and its varieties *compacta*, *carnea*, and *uva* under certain circumstances of growth. The review of the literature on the fungi of sewage by Cooke (1954) indicated that *Sphaerotilus natans* is widespread in sewage and polluted water, that it is known in several forms referred to in the literature as species and even genera, and that it can assume a zoogloeal stage in its life history. Johnson reported on the appearance of seven fungi on trickling filters but the nomenclature he used, while probably valid at that time, refers now to such different organisms, or organisms listed today under such different names, that to repeat them would only add to confusion.

In studies on the fungus populations of trickling filters, both in connection with the technique of sampling such habitats with glass slides (Cooke 1958) and with sampling the populations by scraping the slime from stones (Cooke and Hirsch 1958), 90 species of fungi have been presented. Of these more than 20 species are considered to be members of the permanent population of fungi found on trickling filters.

During much of the year the trickling filter is covered with a green film of algae. This is only one of the several types of organisms in the slime on the upper or surface layers of stone. Except for those algae which are washed down through the filter and somehow manage to survive, these organisms are limited in distribution to those portions of the filter exposed to the light. Early listings included the genera *Chlorella*, *Stigeoclonium*, *Euglena*, *Phormidium*, and *Oscillatoria* without regard to the species represented. In a recent paper by Cooke and Hirsch (1958), the following species were listed from the Dayton, Ohio, trickling filters: *Nitzschia palea*, *Stigeoclonium nanum*, *Ulothrix tenuissima*, *Characium* sp., *Chlorococcum humicolw*, *Chlorella vulgaris*, *Phormidium uncinatum*, *Amphithrix janthina*, *Anacystis montana*, and *Pleurocapsa* sp.

It was found that high-rate and low-rate filters supported different populations of algae especially when loadings were distributed over different periods of time. At Dayton, the high-rate filters receive settled sewage continuously throughout the 24 hours of the day while the low-rate filters received only that in excess of the high-rate filter capacity. Where leakage occurred around the base of the column supporting the distributor arms, the pattern of slime growth is similar on both filters. Diatoms and green algae predominated on the high-rate filters while blue-green algae were abundant on the low-rate filters.

Extensive work on the protozoan and other animal life in the sprinkling filter beds at Plainfield, New Jersey, was published by Lackey (1926). During the course of his study it was found that 19 rhizopods (Sarcodina) occurred in the microbial film on the filter stones. Stones from 1-, 3- and 5-foot depths were examined and the commoner species quantitated. The following rhizopod species were listed: *Actinophyrys sol*, *Actinosphaerium eichhornii*, *Amoeba proteus*, *A. radiosa*, *A. verrucosa*, *Arcella dentala*, *A. vulgaris*, *Centropyxis aculeata*, *Chlamydophrys stercorea*, *Cochiiopodium bilimbosum*, *Difflugia pyriformis*, *Euglypha alveolata*, *Hartmanella hyalina*, *Pamphagus nutabilis*. *Protamoeba primitiva*, *Trinema lineara*, *Vahlkampfia albida*, *V. guttula*, and *V. limax*. Lackey's earlier paper on filter bed protozoans in New Jersey (1924) listed the following genera of rhizopods as including the commoner species: Amoeba, Arcella, Difflugia, Euglypha, and Trinema.

In addition to work on rhizopods, Lackey (1924) listed the various genera of flagellates and ciliates which occurred on New Jersey filters. In addition to "small flagellates" in the Mastigophora, the genera, Distigma, Euglena, Heteronema, and Notosolenus were listed as including at least one species each. In most work with rhizopods, flagellates, and ciliates in the United States the organisms are listed only according to genus. Such listing indicates a lack of interest in identification below the generic level, or difficulties in making specific identifications which would require more time than is available for this type of study.

The genera of ciliates (Infusoria) as listed by Lackey (1924) included: Opercularia, Aspidisca, Blepharisma, Chilodon, Colpidium, Frontonia, Lembus, Lionotus, Metopus, Oxytrichia, Paramoecium, Pleuronema, and Vorticella. In 1926, Lackey added to this list some genera of less common occurrence: Prorodon, Macrymaria, Holophrya, Nassula, Chilodon, Loxodes, Loxophyllum, Amphileptus, Lionotus, Lionotopsis, Glaucoma, Uronema, Colpoda, Colpidium, Microthorax, Lembus, Cyclidium, Pleuronema, Paramoecium; Stentor, Blepharisma, Metopus, Spirostomum; Uroleptus, Amphisia, Oxytrichia, Stylonichia, Euplotes, Aspidisca; Opercularia, Vorticella, and Carchesium. In the Suctoria, only the genus Podophrya was listed. Butcher, in England (1932), has indicated the importance of Carchesium, Vorticella and other stalked ciliates in the biota of sewage by listing them among the organisms commonly called "sewage fungus." Butterfield, Purdy, and Therriault (1931) advanced a theory of the place of these organisms in sewage treatment following a series of experiments at Cincinnati. They stated that . . . "the chief function of certain plankton (Colpidium, a ciliate) in the biochemical oxidation process is to keep the bacterial population reduced below the saturation point and thus to provide conditions suitable for continuous bacterial multiplication, thus in turn resulting in more complete oxidation." Therefore, certain protozoans are seen in both activated sludge and in trickling filter slime as browsing or grazing organisms which may remove some solid, colloidal, or dissolved organic materials, but which primarily aid in the control of the bacterial populations. In some cases, yeast cells may be ingested also but the protozoan organisms are usually too small for effective control of the yeast population.

In a study of the biology of sewage in the Brent and East Ham sewage treatment plants in England, Peters (1930a) listed the protozoans and other animals found throughout the treatment plants. He did not indicate which organisms were found in the trickling filters although 39 of the 52 categories listed were found in them.

Peters (1930b) was especially interested in the nematodes occurring in sewage treatment plants in England. The species Diplogaster strictus, Diploscapter coronata, Dorylaimus saprophilus, and Rhabdites sp. were listed. Workers in the United States, such as Lackey (1924) and Wilson (1943), are content to list nematodes only as a group category without attempting to resolve the populations into the various genera and species which are present. These are among the larger microscopic animals in trickling filter film and any mount will include some individuals.

Among other members of the animal populations of the sprinkling filters at Plainfield, New Jersey, Hausman (1923) listed unidentified nematodes, rotifers, annelid worms including Aeolosoma and Pristina, and Tartigrada. Combined counts at three levels in thousands per cc. of centrifuged film showed some seasonal variation in the occurrence of these organisms.

In 1939, Reynoldson made a very thorough study of (enchytraeid) segmented worms in bacteria beds in England. The most important species was considered to be Lumbricillus lineatus while Lumbricus rubellus was also abundant. Usually adults were found at lower levels while the young were near the surface. Their feeding habitats were closely associated with the growth of Phormidium sp. on the surface film. Drying conditions in summer and cold temperatures in winter tended to send these animals deeper into the filter beds. The cycle of their appearance indicated that they were responsible for sloughing. As the algae returned after 3-4 weeks the worms moved upward to feed upon this growth. The more rapid regeneration of the algae in the summer was also attributed to the effects of the worms. The value of the worms in developing sloughing conditions and in controlling the film was demonstrated experimentally. In the filters under study the worms were considered as important as the insect larvae, Psychoda severini, P. alternata, Metriocnemus longitarsus, M. hirticollis, and Spaniotoma minima, in controlling accumulative film growth. The snail, Limnea glabra, as well as mites and spiders also occurred on these filters.

Wilson (1943, 1949) reported that scouring organisms such as Macrobiotus sp., oligochaete worms, and insect larvae were important in preventing excessive development of film and increasing plant efficiency in Wisconsin and Minnesota. The worms listed included: Aelosoma hemprichi, Pristina sp., and Dero sp. He stated that while worms such as Dero were few in number they exerted a potent influence on the reduction and stabilization of sewage solids and assisted in clearing voids between rocks to the improvement of ventilation.

Imhoff and Fair (1956) indicated that among the worms present in trickling filters were the "aquatic earthworm" Limnodrilus sp., and the reddish bristle

TABLE II. Composite list of trickling filter organisms

(Genera in major groups listed alphabetically regardless of importance or systematics)

- Schizomycetes (bacteria)
  - Sphaerotilus natans
  - Zoogloea ramigera
  - Many unidentified species
- Cyanophyceae (Blue Green Algae)
  - Amphithrix janthina
  - Anacystis montana
  - Oscillatoria limosa
  - Phormidium uncinatum
  - Pleurocapsa sp.
- Eumycophyta (Fungi)
  - Absidia corymbifera
    - cylindrospora
  - Allescheria boydii
  - Alternaria tenuis
  - Ascodesmis microscopicum
  - Aspergillus spp.
    - candidus
    - clavatus
    - flavipes
    - flavus
    - fumigatus
    - niger
    - ochraceous
    - sydowii
    - terreus
    - ustus
    - versicolor
    - wentii
  - Cephalosporium spp.
  - Chaetomium globosum
  - Cladosporium cladosporioides
  - Coniothyrium spp.
    - fuckelii
  - Dematiaceae spp.
  - Epicoccum nigrum
  - Fusarium spp.
    - aquaeductuum
    - oxysporum
    - roseum
    - solani
  - Geotrichum candidum
  - Gliocladium roseum
  - Gliomastix convoluta
  - Leptomitus lacteus
  - Margarinomyces heteromorphum
  - Memnoniella echinata
  - Monilia sitophila
  - Moniliaceae spp.
  - Mucor sp.
    - fragilis
    - plumbeus
    - racemosus
    - saturninus
  - Myrothecium verrucaria
  - Paecilomyces varioti
  - Penicillium spp.
    - brevi-compactum
    - chrysogenum
    - clavigerum
    - cyclopium
    - digitatum
    - expansum
    - funiculosum
    - herquei
    - implicatum
    - janthinellum
    - lilacinum
    - ? luteum
    - martensii
    - nigricans
    - ochro-chloron
    - oxalicum
    - palitans
  - Penicillium spp. (con't)
    - piscarium
    - purpureogenum
    - roquefortii
    - stipitatum
    - variabile
    - velutinum
    - vermiculatum
  - Phoma spp.
  - Pullularia pullulans
  - Rhizopus spp.
    - arrhizus
    - nigricans
    - rhizopodiformis
  - Rhodotorula spp.
  - Sphaeronema spinella
  - Sporotrichum pruinosum
  - Stachybotrys atra
  - Stemphyllium consortiale
  - Subbaromyces splendens
  - Syncephalastrum racemosum
  - Trichoderma viride
  - Trichothecium roseum
  - Verticillium spp.
    - lateritium
  - White yeasts
- Chrysophyceae (Diatoms)
  - Nitzschia palea
- Chlorophyceae (Green Algae)
  - Aeronema polymorphum
  - Characium sp.
  - Chlorella vulgaris
  - Chlorococcum humicola
  - Microthamnion kuetzingianum
  - Richteriella sp.
  - Stigeoclonium nanum
  - Ulothrix tenuissima
- Bryophyta—Musci (Mosses)
  - Leptodictyum riparium
- Protozoa
  - Sarcodina (Amoeboid organisms)
    - Actinophrys sol
    - Actinosphaerium eichhornii
    - Amoeba proteus
      - radiosa
      - verrucosa
      - villosa
    - Arcella dentata
      - vulgaris
    - Centropyxis aculeata
    - Chlamydophrys stercorea
    - Cochliopodium bilimbosa
    - Difflugia pyriformis
    - Distigamoeba gruberi
    - Euglypha alveolata
    - Hartmanella hyalina
    - Pomphagus nutabilis
    - Protamoeba primitiva
    - Trinema lineare
    - Vahlkampfia albida
      - guttula
      - limax
- Mastigophora (flagellates)
  - Anthophysa vegetans
  - Bodo caudatus
    - lens
    - mutabilis
  - Cercobodo sp.
    - longicaudata
    - ovatis
  - Distigma proteus
  - Euglena sp.
  - Heteronema sp.
  - Mastigamoeba sp.
  - Monas sp.
  - Notosolenus sp.

TABLE II. (Continued)

- **Mastigophara (flagellates) (con't)**
  - Oicomonas socialis
  - Peranema trichophora
  - Tetramitus sp.
  - Trepmonas agilis
  - "Many small flagellates"
- **Infusoria (Ciliates)**
  - Amphileptus sp.
  - Amphisia sp.
  - Aspidisca sp.
  - Blepharisma sp.
  - Carchesium sp.
  - Chilodon sp.
    - cucullus
  - Cinecochilum margaritaceum
  - Colpidium sp.
    - striatum
  - Colpoda sp.
    - inflata
  - Cyclidium sp.
    - glaucoma
  - Euplotes sp.
    - sharon
  - Glaucoma sp.
    - scintilans
  - Halteria sp.
  - Holophrya sp.
  - Lembus sp.
  - Lionotopsis sp.
  - Lionotus sp.
    - fascicola
  - Loxodes sp.
  - Loxophyllum sp.
  - Macrymaria sp.
  - Metopus sp.
  - Microthorax sp
  - Nassula sp.
  - Opercu'aria sp.
  - Oxytrichia sp.
    - pellionella
  - Paramoecium sp.
    - caudatum
  - Pleuronema sp.
    - chrysalis
  - Pleurotricha lanceolata
  - Podophrya sp.
  - Prorodon sp.
    - teres
  - Spirostonum sp.
  - Stentor sp.
  - Stylonichia sp.
    - pustulata
  - Uroleptus sp.
  - Uronema sp.
    - marina
  - Vorticella sp.
- **Metazoa**
  - Turbellaria (Flat Worms)
    - Stenostomum leucopus
  - Rotatoria (Rotifers)
    - Euchlanis dilatata
    - Philodina roseola
    - Rotifer vulgaris
  - Nematoda (Nematode Worms)
    - Diplogaster strictus
    - Diploscapter coronata
    - Dorylaimus saprophilus
    - Rhabdites sp.
  - Gastrotricha (Gastrotrich Worms)
    - Chaetonotus sp.
  - Oligochaeta (Round Worms)
    - Aeolosoma hemprichi
    - Dero limosa
    - Limnodrilus sp.
    - Lumbricillus lineatus
    - Lumbricus rubellus

TABLE II. (Continued)

- **Oligochaeta (Round Worms) (con't)**
  - Pristina sp.
    - longiseta
  - Tubifex sp.
- **Polychaeta (Polychaete Worms)**
  - Polychaeta sp.
- **Crustacea**
  - Cyclops sp.
- **Mollusca**
  - Lymnaea humilis modicella
  - Physa anatina
    - cubensis
    - halei
    - integra
- **Tartigrada**
  - Macrobiotus sp.
- **Arachnida**
  - Torrenticula anomala
- **Insecta**
  - Cercyon fimbriatus
  - Diploneura cornuta
  - Leptocera fontinalis
  - Limonia simulans concinna
  - Psychoda alternata
  - Scatella paludum
    - stagnalis

or sludge worm *Tubifex* sp.

Usinger and Kellen (1955) studied the role of insects in several types of sewage disposal beds in California. In an experimental biofiltration plant at San Rafael Meadows insect larvae were found. The commonest species was a member of the Diptera, *Psychoda alternata*. Other Diptera included: *Limonia simulans concinna*, *Diploneura cornuta*, *Scatella stagnalis*, *S. paludum*, and *Leptocera fontinalis*. *Cercyon fimbriatum*, a member of the Coleoptera was also found.

An experimental lath filter was set up in Iowa for the treatment of milk wastes. Frye and Becker (1929) studied the fauna of the slimes which developed on the lath at 1-foot intervals in the 6-foot deep filter. The organisms were presented in a combined list with the protozoans qualified as "common" or "rare." The list included: Protozoa: Sarcodina: Common: *Dimastigamoeba gruberi*, *Vahlkampfia linas*, *V. albida*, *Amoeba* sp., *A. radiosa*, *Euglypha alveolata*, *Chlamydophrys* sp., *Actinophrys sol*, *Centropyxis aculeata*, and *Arcella vulgaris*; Rare: *Amoeba* sp., *A. proteus*, *A. verrucosa*, and *A. villosa*; Mastigophora: Common: *Monas* sp., *Cercobodo ovatus*, *C. longicaudata*, *C.* sp., *Bodo caudatus*, *B. lens*, *Distigma proteus*, *Peranema trichophora*, and *Trepmonas agilis*; Rare: *Mastigamoeba* sp., *Oicomonas socialis*, *Bodo mutabilis*, *Euglena* sp., *Anthophysa vegetans*, and *Tetramitus* sp.; Infusoria: Common: *Chilodon cucullus*, *Colpoda* sp., *C. inflata*, *Cinetochilum margaritaceum*, *Linotus* sp., *L. fasciola*, *Euplotes charon*, *Cyclidium glaucoma*, *Pleurotricha lanceolata*, *Oxytricha pellionella* and *Uronema marina*, *U.* sp., *Vorticella* sp., and *Pleuronema chrysalis*; Rare: *Colpidium striatum*, *Glaucoma scintillans*, *Prorodon teres*, *Stylonychia pustulata*, *Podophrya* sp., *Paramoecium caudatum*, and *Opercularia berberina*; Metazoa: *Turbellaria* (flat worms): *Stenostomum leucopus*; Rotatoria: *Euchlanis dilatata*, *Philodina roseola*, and *Rotifer vulgaris*; Gastrotricha: *Chaetonotus* sp.; Nematoda: unidentified; Annelida: *Aelosoma hemprichi*.

In certain areas, populations of snails can develop in trickling filters. Ingram *et al.* have reported (1958) that at least five species of snails have been found in the United States on trickling filters. *Physa anatina* was found at Urbana-Champaign, Illinois, *Physa halei* at Fort Worth, Texas, *P. cubensis* at Gainesville, Florida, and *P. integra* at Dayton and Hillsboro, Ohio. In addition, *Lymnaea humilis modicella* was found once in low numbers at Urbana-Champaign. Serious damage to equipment was reported as a result of the activities of *P. integra* at Dayton, and of *P. cubensis* at Gainesville.

It is not to be assumed that all organisms presented in the accompanying composite list (Table II) are present on all trickling filters at the same time. Many of them occur in very low numbers indicating that they are transitory rather than members of the population at the time of sampling. Others are very abundant. When Hesseltine (1953) studied the trickling filters at Pearl River, New York, he found that *Fusarium aquaeductuum*, *Geotrichum candidum*, and *Subbaromyces splendens* were common enough to be considered members of the population, and that other species of molds which were present were considered chance contaminants. Becker and Shaw (1955) scraped surface stone of trickling filters at Pullman, Washington, and Moscow, Idaho, and found so few fungi that they decided fungi were not part of the permanent population although *Fusarium aquaeductuum* was present at times in large enough amounts to produce orange colored patches on the filters. Fungi isolated from filter effluents indicated a certain amount of activity on the filters but the extent of this activity can only be guessed on the basis of work done on other filter populations.

Rate of Population Development

Before a complete description of the biota of the trickling filter can be given, information should be available on the development of the population. A method of sampling the algal and fungal components of the population during its developmental and mature stages has been described (Cooke 1958; Cooke and Hirsch 1958). Briefly the technique includes exposure of weighed glass slides on the filter stone, removal of the slides at weekly intervals, and study of the populations developed there. The study is accomplished quantitatively by weighing air-dried materials

TABLE III Fungal species and colonies on exposed slides (first 25 weeks)

Date of Harvest	Week	Number Days Exposure	Low-Rate Filter: Number of Species	Low-Rate Filter: Number of Colonies	Low-Rate Filter: Air dry Weight (gm)	High-Rate Filter: Number of Species	High-Rate Filter: Number of Colonies	High-Rate Filter: Air dry Weight (gm)
11-29	1	7				10	1,662	0.33
12-6	2	14	12	42	0.06	19	989	0.41
12-13	3	21	12	41	0.09	19	740	0.74
12-20	4	28	9	3,190	0.16	17	47,002	0.96
12-27	5	35	12	1,845	0.21	21	48,657	1.40
1-3	6	42	17	35,525	0.39	25	56,694	1.43
1-10	7	49	23	40,463	0.59	23	70,100	1.65
1-17	8	56	19	79,365	0.38	18	174,125	1.35
1-24	9	63	16	124,157	0.81	16	202,025	1.42
1-31	10	70	15	7,696,770	0.88	17	168,175	1.61
2-7	11	77	18	451,675	0.75	23	320,250	0.53
2-14	12	84	22	369,475	0.58	26	344,800	0.69
2-21	13	91	20	564,130	0.64	25	321,055	0.93
2-28	14	98	15	1,988,130	0.78	19	1,473,675	0.64
3-7	15	105	16	2,030,975	0.99	19	832,975	1.43
3-14	16	112	18	16,439,000	1.14	14	3,136,750	1.51
3-21	17	119	14	9,929,750	0.88	16	1,112,400	0.71
3-28	18	126	14	2,731,250	1.78	15	1,654,750	1.05
4-4	19	133	12	9,077,050	0.59	15	6,814,750	1.40
4-11	20	140	14	13,872,000	1.06	18	14,131,250	1.82
4-18	21	147	12	7,161,750	0.87	12	10,921,000	1.75
4-25	22	154	13	5,114,750	0.57	16	12,413,250	2.20
5-2	23	161	12	10,610,000	0.69	15	7,200,000	1.76
5-9	24	168	13	2,730,000	1.10	15	5,575,000	1.78
5-16	25	175	12	13,495,000	1.28	17	7,935,000	1.50

on the slides, by reviving some members of the population, plating them and counting the colonies or by directly counting cells and cell clusters in the materials which have been resuspended in water.

At the Dayton, Ohio, sewage treatment plant, studies were made simultaneously on a low-rate and a high-rate trickling filter. Sixty sets of frames, each with four attached slides, were placed on each filter. Slides were returned to the laboratory at weekly intervals for 57 weeks. Colony counts of fungi found in the rewet material were obtained for each slide from each filter. These are presented in Table III for the first 25 weeks for both filters. A mathematical curve, the logistic, was fitted to the population growth data, that is, colony counts from the start of observation to the time at which these counts appeared to level off. The equation for this curve is:

$$\text{Expected } y_t = \frac{k}{1+e^{-(\alpha+\beta\log t)}} = k\,\frac{e^{\alpha+\beta\log t}}{1+e^{\alpha+\beta\log t}}$$

where y_t is the colony count at time t, measured in weeks, k is the plateau level, determined as the average of the first several colony counts (7 for the low-rate, 4 for the high-rate) after what appeared to be the leveling off time (15 weeks for the low-rate, 19 for the high-rate filter), and α and β are parameters (Fig. 1). For the high-rate filter, k was found to be 12,800,000, and for the low-rate filter, k was 9,200,000.

Estimates of the parameters α and β are obtained by putting the above equation in the following linear form in terms of common logarithms:

$$\text{Observed } \log_{10}\frac{y_t}{k-y_t} = .43429\ (\alpha+\beta\log t).$$

To ease the calculations, the factors $y_t/k-y$ were multiplied by 100,000 in the case of the high-rate filter and by one million for the low-rate filter.

$$\text{Thus, if } \frac{y_t}{k-y_t} := 100{,}000\ \frac{y_t}{k-y_t}\text{, we obtain}$$

$$\log_{10}\frac{y_t}{k-y_t} := 5 + .43429\,\alpha + .43429\,\beta\log t$$

(in the case of one million, the 5 on the right-hand side is replaced by 6). Thus, plotting the observed numbers on the left-hand-side versus log t, a good straight line was obtained on log-log paper whose intercept, say A, equal to 5 + .43429 and whose slope, B, equal to .43429, were estimated by the usual least squares techniques. Then it was estimated that

$$\alpha = \frac{A-5}{.43429} = 2.30259\,(A-5)\text{, and } \beta = \frac{B}{.43429} = 2.30259B$$

For the low-rate filter these estimates were $\alpha = -16.92$, $\beta = 13.25$, and for the high-rate filter $\alpha = -10.87$, $\beta = 7.19$.

Using these values of α and β and the previously mentioned values of k, Figure 1 plots these values on semi-log paper for each filter and shows the observed colony counts and those expected under the fitted logistic curve.

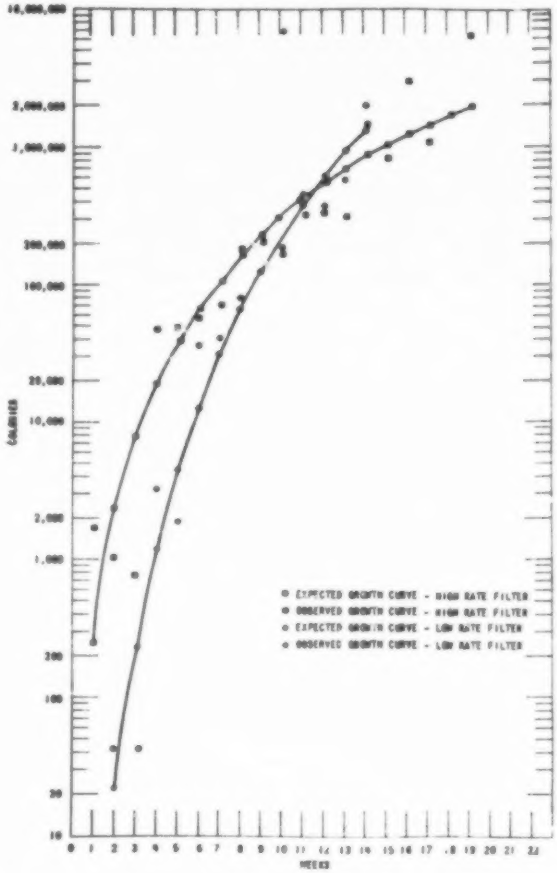


Figure 1 - Colony counts observed and expected under fitted logistic curve.

It will be noted that the population growth curve rises more sharply and steeply on the low-rate than on the high-rate filter and would arrive at the upper asymptote, k, more quickly. In view of the fluctuations observed during later weeks, it is believed that there is, in the long run, no real difference between the two selected values of k. The logistic appears to be an adequate representation of the increase in the number of colonies during the build-up phase.

Description of the Biota

The community of organisms occupying a trickling filter, and the changes through which it goes as it develops from its inception or as a result of changes in dosing or in quality of applied sewage, can be described in terms of the formulae given by Major (1951). His basic formula can be modified for application to this type of population as follows:

$$b = f_b\,(cl, p, r, o, t)$$

where b, the biota of a specific trickling filter, is a function of cl, the regional climate, p, the physical and chemical quality of the applied sewage at time zero, r, the fixed operational conditions of the specific, o, the biotic effect of the organisms of the community and of man, and t, the time.

At any moment in time the biota of the filter becomes adapted to conditions of growth on the filter through natural selection rather than deliberate seeding of special segments of the population. The most important parts of this equation are thus p and o. The kind of sewage p applied to a filter will determine the members of the population, since some species will not be able to tolerate some of the sewage materials because of toxicity or inability to use them as food. The factor of man's interference o with the population is important since dosing rates may vary, different types of sewage may be applied at different times, and other operational problems may interfere with one or another type of population. Factors of regional climate cl will be relatively constant within one filter although they may vary between different parts of the country or between different types of filter. Within one filter, or one filter field, the regional climate will not vary and will not be particularly important except to the immediate surface layer. Within one filter, temperature of applied sewage will be relatively constant and will be affected only slightly by air temperature. Precipitation in the form of applied sewage will be constant except during periods of heavy rainfall when some of this water is diverted to the filters and is received in the form of more dilute sewage applied more rapidly than usual. Effects of such dilution are usually of brief duration and not of catastrophic importance. Some sloughing may result but recovery is relatively rapid. Effects of the r type would be noticed on the filter by the differing populations on the surface layer of stone, because of the presence of light-sensitive algae and fungi, as contrasted with those populations at lower levels. Types of filter construction, ability to prevent ponding, and rate of application of sewage so far as diameter of distributor arms and stationary nozzles are concerned, would be noted in variations of r-type effects. Effects of time t would vary only slightly if the sewage dosage throughout the year were relatively constant in composition and rate of application, and if the sewage were applied to a well designed and constructed filter. That is, throughout the year, one could expect a certain type of biota to develop on a trickling filter under relatively constant conditions of regional climate, including normal seasonal fluctuations, relatively uniform municipal or industrial waste applications, relatively uniform and continuous operation of the filter, and relatively uniform development of the population, including growth, death and replacement of component parts, without undue interference from man.

Variations in populations occur when dosing rates are changed, type of sewage applied is altered chemically or physically, filters are stopped for servicing, flooding conditions are induced by excessive precipitation or in attempted control of insect or snail populations, or when other factors are altered. Changes in population patterns can be noted by casual macroscopic observation when excessive sloughing occurs, but such observations are not useful unless accompanied by detailed analyses of all aspects of sewage influents and effluents and of engineering factors involved.

Activities of Segments of Filter Bed Populations

It has been suggested, in connection with the listing of the several groups of organisms, that during the course of their life on or in the microbial film these organisms contribute to sewage purification. Upon and within each filter bed a balanced population develops. The population of the film is built up gradually, although the time required may be as short as 50 days (Gilcreas and Sanderson 1940). Its members are derived from various sources and received by the filter either from influent sewage, from initial seeding by the use of effluents from other trickling filters, or by settling of disseminules from the air upon the filter. Such members of the raw sewage population as are readily adapted to life on the filter settle out and become attached to the filter stone. If they can successfully compete with the population already present they will become members of the population of the microbial film.

The algae derive some mineral and nitrogen nutrition from the sewage and are close to an ample supply of carbon dioxide. In turn, they add oxygen to the water in which they are continually bathed. Bacteria and fungi are able to remove quantities of solid, colloidal, and dissolved organic materials from the water through metabolism and by supplying a film onto which many of these materials can be adsorbed and thus effectively removed as the sewage passes through the filter. Protozoans and smaller metazoans easily devour large numbers of bacteria, thus removing members of that population and making space in that particular ecological niche for additional bacteria. This activity tends to control bacterial multiplication keeping it in a continual logarithmic growth phase. If the organism has a large enough "mouth," yeast cells may also be ingested and a similar control is effected on the yeast population. The larger metazoa, such as annelid worms, insect larvae, and snails, browse on the larger fungal and algal populations. Although they are not necessarily carnivorous, the metazoa may ingest, at the same time, any of the smaller organisms and sludge materials adsorbed upon the biological film on which the larger metazoans are feeding.

The concluding statement by Ingram et al. (1958) may be elaborated as follows: In removing organic slimes through feeding, snails and annelids remove some percentage of B.O.D., as do various insect larvae. They can thus be looked upon as "crawling B.O.D. removed," as Psychoda and other flies and insects may be visualized as representing "flying B.O.D. removed." While the efficiency of this removal has never been evaluated satisfactorily, it is indicated by the large numbers of filter flies around trickling filter installations, and by the snails, as many as a ton, that can be removed from a trickling filter plant in a day. In terms of food chains, annelid worms, insect larvae, and snails represent the terminus of production on the trickling filter.

Using chemical and biological analyses of materials on a new trickling filter in New York State, Gilcreas and Sanderson (1940) were able to determine that there was a gradual increase in the degree of oxidation of organic and nitrogenous matter in settled domestic sewage at the temperatures received coincident with the gradual development of microbial populations. As a result of a study conducted during the first 107 days of operation it was found that complete oxidation of nitrogenous compounds was effected after 40 days, 50 days were required for total development of the characteristic biota of trickling filters, and 60 days were required for maximum reduction of carbonaceous organic matter.

C. E. Renn (1956) stated: "I am inclined to prefer Dr. Phelps' view of the role of the trickling filter to the more conventional conception of high oxidation rates on surfaces. We have no comparable experience with other types of surfaces that justifies the extraordinarily high rates of oxidation that are implied by assuming that the B.O.D. of a waste is removed in the interval of liquid passage. Rather, there is a great deal of evidence to show that we accumulate a large stock of organic material locally, through high rates of adsorption, and that the accumulated organic materials are oxidized at rates comparable to those of other organic systems."

Activities of the Microbial Film

A trickling filter is so designed that a large amount of biological film is exposed to the flow of sewage. Depending on plant design, this liquid material has passed through preaeration and settling tanks, as at Dayton, so that a portion of the solid, colloidal, and dissolved organic materials has been acted upon and settled out before the liquid reaches the filters. However, a considerable amount of dissolved, colloidal, and solid material remains. As the liquid passes over or through the film, some of these materials are removed through metabolic processes if microorganisms and sometimes of macroorganisms, and additional portions are removed by adsorption on the film. The organic nitrogen content is a measure of the decomposable materials present at any time or place and is used together with B.O.D. as an indicator of the purification effected within a process.

Is it possible to relate the extent to which the removal of organic matter and the mineralization of various constituents of sewage materials is effected by the organisms of the microbial film to the numbers and types of organisms on the surfaces of the filters at the Dayton treatment plant?

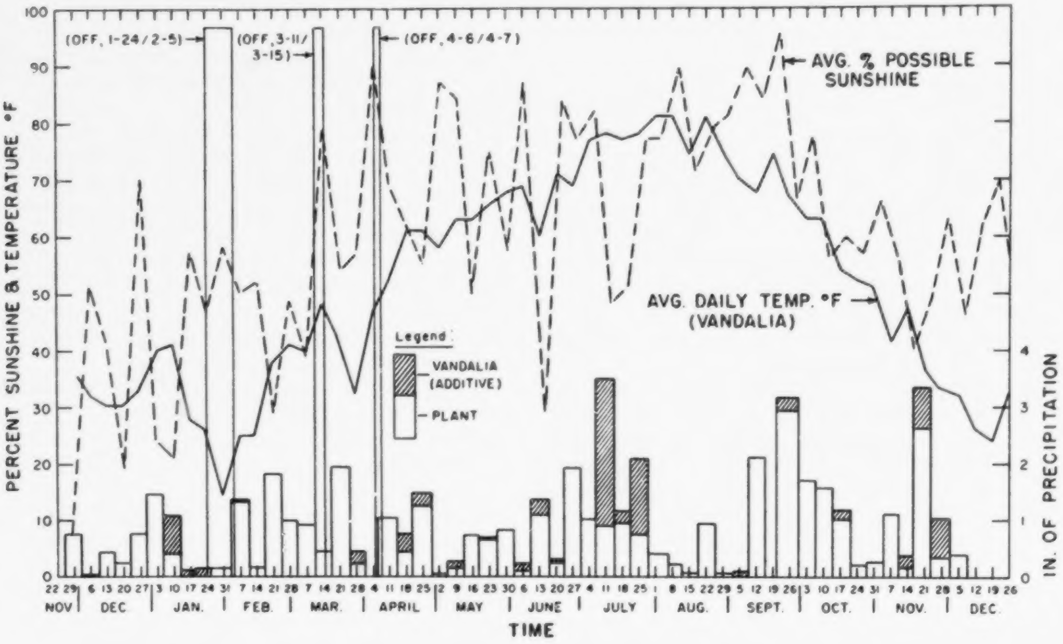


Figure 2 - Weekly averages of average daily temperature, average percentage possible sunshine, and total effective precipitation for the Dayton area.

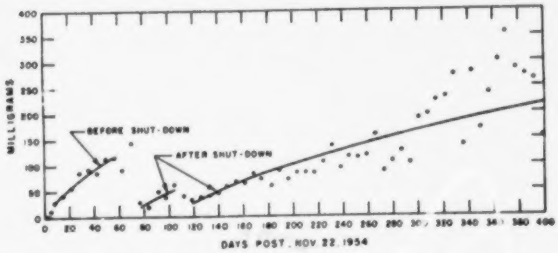


Figure 3 - Cumulative growth on frames showing influence of shut-downs and rate of development.

Some idea of the potentialities of this question may be gained from the fact that a single trickling filter, 165 feet in diameter, 7.5 feet deep, is filled with 160,270 cu. ft. of limestone slag rock of varying sizes, usually 3-5 inches in diameter, length or width. A flat circular surface 165 ft. in diameter comprises an area of 20 million sq. cm. The upper surface of the stone on a trickling filter is even more extensive because of the irregularities of the stone. The sampling technique included 75 sq. cm. of glass slides on which determinations were made at weekly intervals. Such sampling took into consideration only the initial contact of the sewage with the microorganisms and did not consider the subsequent flow of sewage through a 7.5 foot column of rock. Levine (1938) has shown that as sewage passes downward through the filter the nature of the materials in it become progressively different resulting in gradual downward change in the biota acting upon it.

Preaeration tanks were placed in service at the plant in July 1954, but the complete effect of this type of early stage treatment, preceding primary settling, was not felt on the filters until the following May. This delay may have resulted from the further complication that the filter beds were temporarily by-passed, because of repair shut-downs for three periods between January and April. Throughout the study periods, the high-rate filters took a maximum load of sewage while the low-rate filters received only the excess. Materials entering any of either type of filter were not metered, nor is there a record of the hours or of days during which the low-rate filters were not operating. Low-load periods occurred on weekends and during the night. Since effluents from the two types of filters were not kept separate, data for filtered sewage represent a composite from both types. On the other hand, only data from the slides exposed to the surface of a high-rate filter are used in comparing results obtained on analysis of filtered sewage composited from both filters. On the basis of such data, and because only the surface of the beds was sampled, it may be surprising to find any correlation between air-dry weight of organisms and amount of activity produced by such organisms.

Using available climatic and plant records (Fig. 2), an attempt was made to determine whether the average weekly air temperature (Vandalia, Ohio), the average monthly sewage temperature, and the total weekly precipitation (sum of the higher of the two daily recordings from Vandalia and the sewage treatment plant) showed correlation with the amount of growth on the slides as indicated by average weights of material on four slides from each of the high- and low-rate filters.

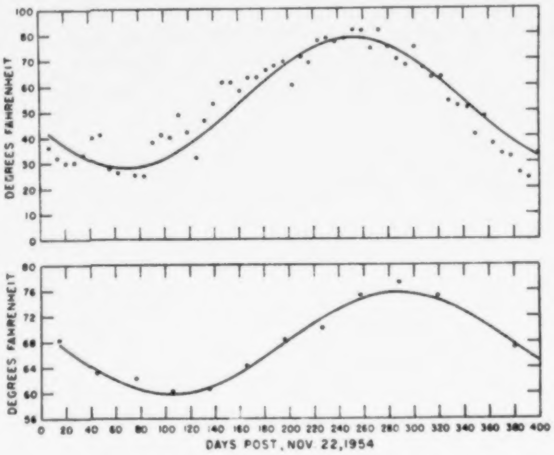


Figure 4 - Average weekly air temperature (Vandalia, Ohio) and average monthly temperature of raw sewage (Dayton).

Weight per slide. - Certain discontinuities in the growth curve were traced to periods when the plant was shut down, or the filters by-passed. Enough time was subtracted from those periods associated with readings following shut-downs to make the weight on the slides immediately following the shut-down coincide with the weight of the original build-up preceding the shut-down. It was found that growth following a shut-down was noticeably slower than that preceding a shut-down when the same weight of material occurred on the slides (Fig. 3). Straight-line curves fitted to the data on log-log paper show that the percentage growth rate is inversely proportional to time. Growth during the first month of exposure (before the first shut-down) was about 61 mg. per 30 days, but after the shut-downs, the growth rate was only 35 mg. per 30 days when the same weight (25 mg.) of organisms occurred on the slides. On this basis, an equilibrium had not been reached at the end of the exposure period since the growth rate was 17 mg. per 30 days during the 12th to 13th month.

Average weekly air temperature (at Vandalia). --A trigonometric sine function was fitted (Fig. 4) to these data successfully. The minimum (trough) value of the fitted curve is 28.2° F. and occurred in the 10th week of the experiment (ending Jan. 31, 1955). The maximum (peak) value of 78.2° F. occurred six months later in the 36th week (ending Aug. 1, 1955). The rise from the trough to the peak is a mirror

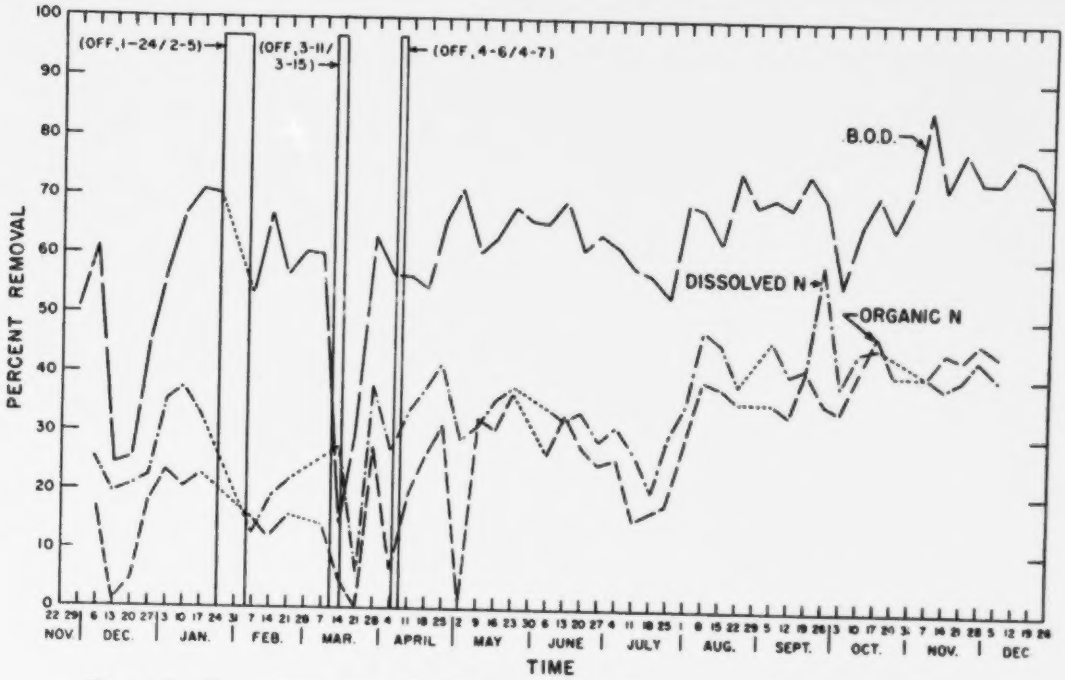


Figure 5 - Percentage removal of B.O.D., dissolved nitrogen and organic nitrogen during filtration.

image of the drop from the peak to the next year's trough.

Average monthly raw sewage temperature. --A sine curve (Fig. 4) also fitted these data well. The minimum [illegible] 59.7 F. lay in Feb. 1955, and the maximum of 75.6 F. lay in Aug. 1955. The sewage temperature curve lagged the air temperature curve by about 40 days, but since the sewage temperatures were monthly averages and air temperatures were weekly averages, the lag could have been as little as three weeks.

Total weekly precipitation (sum of the higher of two daily recordings). --(Fig. 2) There was no trend in the average weekly precipitation during the year. Weekly precipitation apparently varies at random an average of 0.90 inches per week, but is not symmetrically distributed about this average. That is, there were more values below the average (35) than above (22).

Correlations. --Several statistical techniques were tried to determine whether or not the microbial film was affected by the climatic conditions to which it was subjected throughout the year. No evidence exists to indicate a relationship between the irregularities (sloughing or unusually rapid growth) in slide weights and corresponding deviations from seasonal patterns of the weekly average air temperature, raw sewage temperature, or total weekly precipitation figures.

Chemical and physical analyses of sewage.--A chemical and physical check is maintained by the chemical laboratory of the Dayton sewage treatment plant on the effectivity of the treatment. At four stages in the treatment process--raw, settled filtered and final--the B.O.D. , dissolved nitrogen, ammonia nitrogen, organic nitrogen (Fig. 5, expressed as percent removal), chlorides, and suspended solids (Fig. 6) are analyzed. The 5-day B.O.D. and suspended solids are measured daily at each of these points. The other values are taken on the 1st, 8th, 15th, 22nd, and 29th of each month unless one of these dates happens to fall on or near a holiday. On the accompanying graphs, values for these factors are plotted as near the day on which sampling was done for the week as possible. Values used in the present study include only those for settled sewage entering the filters through the distributor nozzles, and filtered sewage, as it leaves the filters before it reaches the final settling basin.

Correlations.--Of the five chemical factors measured in the settled sewage, only the B.O.D. was related to variations in the growth rate of organisms occurring in the slimes with any degree of significance, and even the B.O.D. fails to explain any but an insignificant portion of the variability in the growth pattern of the organisms on the surface of the filter. Most of the statistical analyses of the effect of the chemicals on growth of microorganisms and of the organisms on the chemicals were made with the use of regression coefficients. No significant regression coefficients of slide weights them-

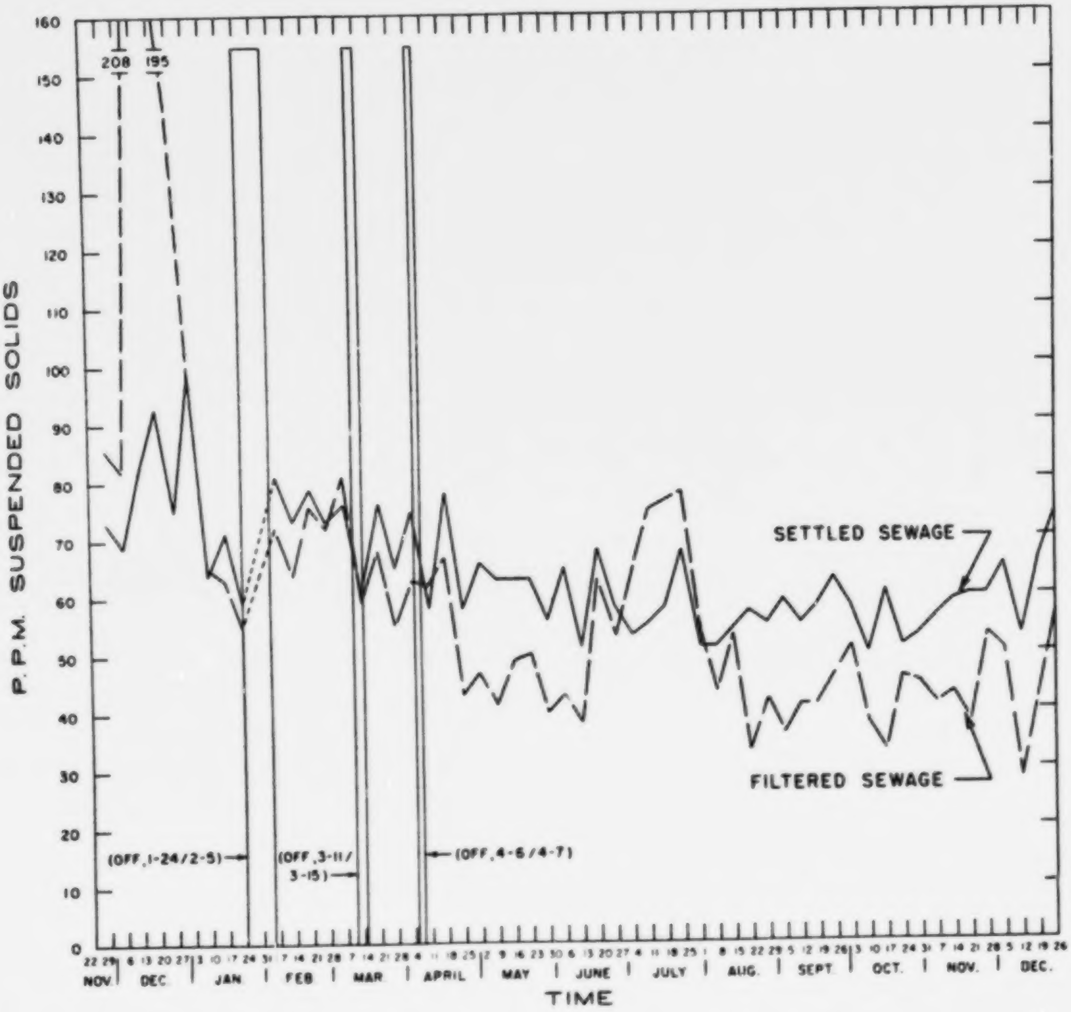


Figure 6 - Suspended solids in settled and filtered sewage.

selves were noted on the chemicals in the settled sewage.

Regression coefficients were found to be satisfactory for assessing the possible dependence of the chemical concentrations in the filtered sewage on the weight of organisms on the slide surfaces. Using this technique it was found that the differences between the B.O.D., organic nitrogen, and dissolved nitrogen in settled and in filtered sewage were related to slide weights. Thus these differences can be postulated as attributable to the removal from sewage of materials, adsorbed or as increased growth, added to the weight of organisms present. Such slide weights represent neither increment nor total accumulated growth but material present at any one date following addition by growth and adsorption, as well as loss by death and sloughing. On the other hand, the amounts of ammonia nitrogen and suspended solids in either the settled or filtered sewage did not appear related in any way to the organisms on the surface of the filter. In the case of suspended solids, this observation is of interest since a good proportion of these solids is composed of sloughing from the microbial film.

Adaptation to Habitat

The organisms which live in the sewage treatment plant, especially those on the trickling filters, have become adapted to life in a waterfall-cascade system in which the effluent from primary settling tanks is the liquid passing over the rocks. This liquid contains a quantity of suspended, colloidal, and dissolved materials, primarily waste materials resulting from human metabolism but also including such wastes as laundry and wash water, industrial wastes, soil run-off, and wastes from garbage disposal units. Fundamentally, there is little difference between a habitat in which such materials are present and a soil habitat which contains fragments of dead plant or animal parts, as well as the excrement of various native animals. Many, if not all organisms which live on trickling filters have become adapted to this condition of existence following the development of a capacity to live all their lives in the presence of water.

According to various interpretations of the Kolkwitz and Marsson (1911, 1950) system of classification of zones of pollution in streams (Whipple, Fair and Whipple 1947), the solids removed from sewage during the settling process and treated in sludge disgesters form a *polysaprobic* habitat while the settled liquid passing over the trickling filter provides a habitat interpreted as *a-mesosaprobic*. Thus all of the organisms found living in a trickling filter (Table II) could be interpreted as *a-mesosaprobes*. However, there appear to be different degrees of adaptation to such an environment. Cooke (1957a, 1957b) has indicated that for the fungi there may be four different types of response to the pollutional habitat. The terminology defined in these papers is not necessarily restricted to fungi but may be applied to all organisms living in the habitat. Those species which have never been reported except from trickling filters, dung, decaying animal bodies under water, and similar habitats may be considered *lymabionts*. At least three species of fungi (*Pilobolus* sp., *Ascodesmis microscopicum*, and *Subbaromyces splendens*), *Tubifex* worms, and possibly some bacteria and protozoans may be included in this category.

Most of the organisms found on trickling filters may be considered as *lymaphiles*. They are closely associated with this type of habitat although in certain instances they may be found in other habitats where decay occurs or where conditions are tolerable.

Of the fungi, at least, a large number of species, represented by a small number of individuals, are considered to be *lymaxenes*. These organisms are well adapted to living in habitats other than those which may be considered sewage polluted, but for short periods of time they can join the other members of the trickling filter population. At such times they may be passing through the plant as chance contaminants and become trapped in the slime by adsorption or by mechanical screening methods.

One will never find a *lymaphobe* in this habitat at least in vegetative condition. Such a find would be the result of a chance contamination, a spore being carried through the plant by the water in which it was accidentally picked up.

While much of the biology of algae, protozoans, metazoans, and bacteria which are found on the filters as members of the population is not well known, it may be assumed that these organisms, too, will be found to fall into the biological classes listed above. Trickling filters, as such, have been in existence for hardly 80 years (Stanbridge 1954), and it does not seem likely that a whole new population of organisms could have developed during that time. It seems more probable that members of other populations have become adapted during this period of time to life in this specialized habitat. Prior to the formal recognition of the need for sewage purification in the late nineteenth century, sewage disposal or removal was a more or less individual matter and where sewer lines existed they only carried the wastes to the nearest streams. Certain members of the population of organisms adapted to a sewage habitat may have originated from the biota of such waste-carrying channels.

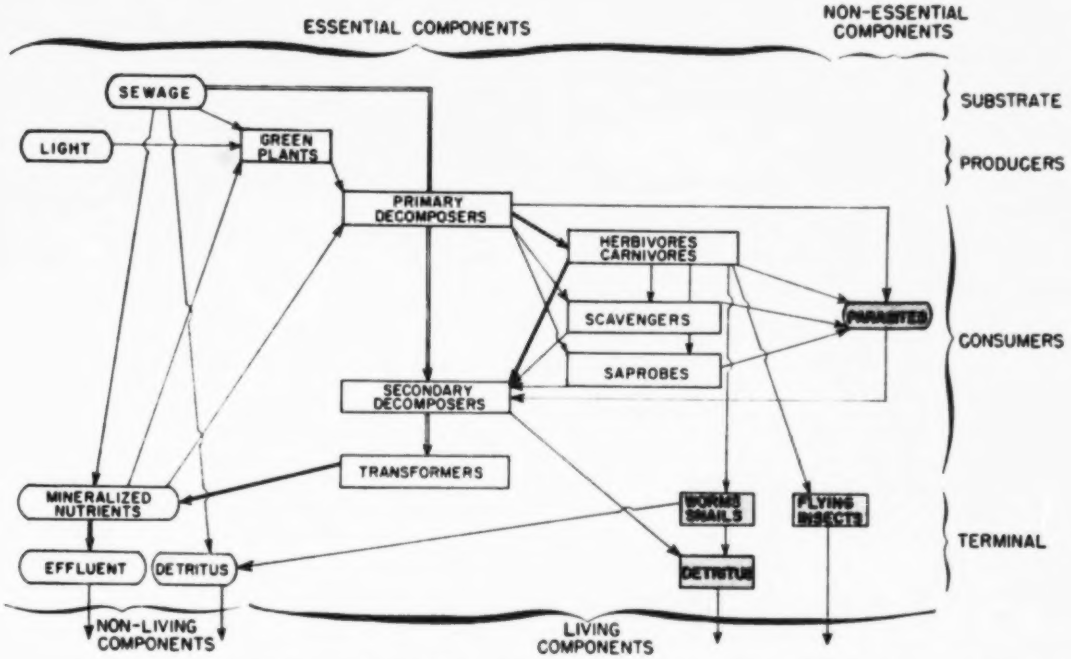
The Ecosystem

The ecosystem of the trickling filters may be summarized by the chart presented in Figure 7. This has been adapted from Clarke (1954) whose original chart (p. 467) has been modified to describe the specialized ecosystem under consideration. In this chart it is assumed that the trickling filter whose population is being described is not covered and that direct sunlight is being supplied to the algae which are the only green plants functioning in the treatment of sewage. It is indicated that the sewage being applied is an essential component since the population is developed for the treatment of this material. A somewhat artificial pair of categories has been introduced by the splitting of "decomposers" into primary decomposers, those which act directly on the sewage components, and secondary decomposers, those which act on dead tissue formed through development of the biological film.

From sewage, green plants can obtain certain required mineral supplies as well as nitrogen and phosphorus so that in the presence of light growth can be effected. It is possible that within the microenvironment there is present a large amount of carbon dioxide than in many habitats because of the great numbers of microorganisms existing in an actively metabolizing state. Those green algae which occur on the filter, then, are probably conditioned to a slightly higher percentage of atmospheric carbon dioxide than other algae or than algae of the same species in a different aquatic habitat.

In some cases it is difficult to define the exact meaning of the terms herbivores and carnivores as related to the organisms on the filters, because of the problem of separating plants and animals in a population of this type. In some cases, pigmented cells, unpigmented cells and nonliving particulate matter all form the basis of the food supply of grazing and scouring organisms.

Herbivores and carnivores include protozoans and metazoans which feed only in part on bacteria. These organisms thus exercise a control on the rate of growth of the film and on the amount of growth which may be present. *Colpidium* and other ciliates keep



PRINCIPAL STEPS AND COMPONENTS IN THE TRICKLING FILTER ECOSYSTEM

Figure 7 - Principal steps and components in the trickling filter ecosystem.

the bacterial populations in a continuing state of growth by ingesting bacterial cells and permitting the continued rejuvenation of that segment of the biota. In addition, other protozoans may act in a similar way and larger animals, such as rotifers, may ingest larger unicellular organisms including algae and yeasts with similar effects. Insect larvae, sludge worms and snails graze on the film. These organisms are probably both carnivores and herbivores since they may ingest both pigmented and unpigmented cells, bacteria, fungi, algae and protozoans. Adsorbed on the less motile components of the film are sludge deposits of various types which may be ingested together with the film so that in a sense these browsing and grazing organisms are scavengers and saprobes as well.

It is possible that some members of the population are parasitized by some microorganisms. Such relationships have not been shown directly, but in the examination of algal material submitted to him for identification Drouet (in Cooke and Hirsch 1958) indicated that *Pleurocapsa*-like material could have been a parasitized form of an *Anacystis*-like species. *Characium* sp. was found growing attached to *Stigeoclonium nanum* but this was probably an epiphytic relationship. While a number of types of bacteria and fungi are known to be able to attack members of the trickling filter biota in other habitats, parasitic relationships between the members of this biota and each other or additional invaders have not been observed. Rather, a mutualistic relationship has been established especially between the unicellular colonial organisms and the filamentous organisms in which the byproducts of the metabolism of one type of species are removed by another thus reducing potential toxic effects.

Scavengers and saprobes may degrade the dead tissues of the primary decomposers as well as components of the sewage but in turn they are subject to decomposition by the secondary decomposers whose byproducts are transformed into mineral nutrients which may also be added to the influent sewage to help restart the cycle.

SUMMARY AND CONCLUSIONS

The trickling filter is an ecological tool of the sanitary engineer in the treatment and purification of domestic sewage and industrial wastes and terminology developed in its use is described. The biota of trickling filter slime has received little attention as a working population unit although various segments of that unit have been described.

The populations of several trickling filters in the United States and England are reviewed according to limited studies based on either bacteria, fungi, algae, protozoans, worms, insects or snails. An integrated list is presented to indicate something of the complexity of the biota.

A series of slide exposure samples of trickling filter biota has been studied with special reference to algae and fungi. As a result of fungal colony counts made from revived growths, a logistic curve has been developed to describe the rate of development of the population. The more rapid and steeper rise of the curve on a low-rate than on a high-rate filter is more apparent than real since the same curve is obtained in plotting the lower values of the two curves in parallel, so that no actual difference was noted between the two types of filter.

The functional, factorial description of a community can be used to describe the biota of a trickling filter. Climate is relatively uniform for a single filter except in periods of excessive precipitation or run-off. The biotic effects within the community are limited to replacement phenomena should an organism be unable to meet competition, and to the fact that grazing organisms tend to control the rate of population growth. Operational conditions of a specific filter, man's effect on a filter as operator and controller of the process, and the physicochemical nature of the applied sewage would be effective in time, but at the end of a study of samples accumulative up to one year the effect of time itself had not been completely discovered.

Filter bed populations are effective in removing a large percentage of solid, colloidal, and dissolved matter in settled sewage, thus reducing the B.O.D. of that sewage. This removal is not by metabolic processes alone but by adsorption on the film as well.

Growth on the stones of the filter develops rapidly at first but slows down as the film matures. In the case of reported experiments, points at which such slowing was most noticeable coincided with periods during which the filters were shut down for repair. No correlation between surface growth rate and total weekly precipitation, average weekly air temperature, and average monthly sewage temperature was noted. Surface growth was not correlated with differences between settled and filtered sewage of ammonia nitrogen, organic nitrogen, chlorides or suspended solids. However, a slight correlation was noted in the case of B.O.D. removal.

As a whole, the habitat is a-mesosaprobic. However, different component organisms vary in their ability to tolerate the conditions to which they are exposed. Species represented most abundantly are lymaphiles. Many species represented by few individuals are lymaxenes, while the lymabionts have not yet been noted elsewhere. Lymaphobes are not expected in this habitat although an occasional spore or resting body may be observed.

The total ecosystem includes a few photosynthetic algae but mostly organisms dependent on preformed organic matter. Primary decomposers use such materials as food supplies, may be acted on by herbivores or carnivores or may be parasitized. At death all organisms of the system may be attacked by scavengers or saprobes which act as secondary decomposers whose products are transformed into mineralized nutrients which may reenter the metabolic system.

ACKNOWLEDGEMENTS

Assistance in laboratory studies has been given by George Matsuura. In statistical analyses, Kenneth A. Busch and Eugene K. Harris gave much help. P. W. Kabler, Ernest C. Tsivoglou, William M. Ingram, Archie E. Becher, and Graham Walton of the Sanitary Engineering Center helped in clarification of sections of the paper. George C. Clarke, Harvard University, kindly criticized the development of the ecosystem and aided in the clarification of the ecosystem chart. R. F. Daubenmire, The State College of Washington, also contributed to clarification of certain passages.

REFERENCES

Amer. Soc. Civil Eng. 1949. Glossary, Water and Sewage Control Engineering , pp. 274. ASCE, New York.

Becker, J. G. 1953. A study of fungi in domestic sewage treatment plants. Thesis. The State College of Washington, Pullman.

-------- and C. G. Shaw. 1955. Fungi in domestic sewage treatment plants. Applied Microbiology 3: 173-180.

Butcher, R. W. 1932. Contributions to our knowledge fo the ecology of sewage fungus. Trans. Brit. Myc. Soc. 17: 112-124.

Butterfield, C. T., W. C. Purdy and E. J. Therriault. 1931. Experimental studies of natural purification in polluted waters. IV. The influence of the plankton on the biochemical oxidation of organic matter. Pub. Health Repts. 5, 46: 393-426.

Clarke, G. L. 1954. Elements of Ecology, pp. xiv, 534. John Wiley & Sons, Inc., New York.

Clark, H. F., E. E. Geldreich, H. L. Jeter and P. W. Kabler. 1951. The membrane filter in sanitary bacteriology. Pub. Health Repts. 66: 951-977.

Cooke, W. B. 1953. Mosses in a sewage treatment plant. The Bryologist 56: 143-145.

-----. 1954. Fungi in polluted water and sewage. I. Literature Review. Sewage and Industrial Wastes 26: 539-549.

-----. 1957a. Check-list of fungi found in sewage and polluted water. Sydowia, Annales Mycologici Ser. II., Beiheft 1: 146-175.

-----. 1957b. Use and value of fungi as biological indicators of pollution. pp. 84-93 in Biological Problems in Water Pollution. Robert A. Taft Sanitary Engineering Center, Cincinnati.

-----. 1958. Continuous sampling of trickling filter populations. I. Procedures. Sewage and Industrial Wastes 30: 21-27.

-----. 1958. Fungi in polluted water and sewage. IV. The occurrence of fungi in a trickling filter-type sewage treatment plant. In press.

----- and A. Hirsch. 1958. Continuous sampling of trickling filter populations. II. Populations. Sewage and Industrial Wastes 30: 138-156.

Frye, W. W. and E. R. Becker. 1929. The fauna of an experimental trickling filter. Sewage Works Journ. 1: 286-308.

Gilcreas, F. F. and W. W. Sanderson. 1940. The development of sewage oxidation by a trickling filter. Sewage Works Journ. 12: 73-80.

Hausman, L. A. 1923. Studies on the fauna of the sprinkling filter bed and Imhoff tanks. New Jersey Agr. Exp. Sta. Bull. 390: 28-39.

Hawkes, H. A. 1952. The ecology of *Anisopus fenestralis* Scop. (Diptera) in sewage bacteria beds. Ann. Appl. Biol. 39: 181-192.

Hesseltine, C.W. 1953. Study of trickling filter fungi. Bull. Torr. Bot. Club 80: 507-514.

Heukelekian, H. and E. S. Crosby. 1956. Slime formation in sewage. III. Nature and composition of slimes. Sewage and Industrial Wastes 28: 206-210.

Hotchkiss, M. 1924. Studies on the biology of sewage disposal. A survey of the bacteriological flora of a sewage treatment plant. Jour. Bact. 9: 437-454.

Imhoff, K. and G. M. Fair. 1956. Sewage Treatment. pp. vi, 338. John Wiley and Sons, Inc., New York.

Ingram, W. M., W. B. Cooke and L. J. Hagerty. 1958. Operational difficulties caused by snails in sewage treatment plants. Sewage and Industrial Wastes 30: 821-825.

Johnson, J. W. H. 1914. A contribution to the biology of sewage disposal. Journ. Econ. Biol. 9: 105-124, 127-164.

Kabler, P. 1957. Use and value of bacterial indicators of pollution. Biological Problems in Water Pollution. Robert A. Taft Sanitary Engineering Center, Cincinnati. pp. 43-49.

Kolkwitz, R. 1950. Okologie der Saprobien. Über die Beziehungen der Wasserorganismen zur Umwelt. Schrift, Ver Wasser-, Boden-, and Lufthygiene 4: 1-64.

Kolkwitz, R. and M. Marsson. 1911. Biologie des Trinkwassers, Abwassers und der Vorfluter. Rubner, Gruber, and Ficker's Handbuch der Hygiene II. 2 S. Hirzel, Leipzig.

Lackey, J. B. 1924. Studies on the fauna of Imhoff tanks and sprinkling beds. New Jersey Agr. Exp. Sta. Bull. 403: 40-60.

-----. 1926. Sprinkling filter bed studies. New Jersey Agr. Exp. Sta. Bull. 427: 41-50.

Levine, M. 1938. Bacteriological aspects of sewage treatment in Modern Sewage Disposal, New York. pp. x, 371 (139-157).

Major, J. 1951. A functional, factorial approach to plant ecology. Ecology 32: 392-412.

Orford, H. E. 1957. Preliminary report on recommended units of expression for design and operation of sewage and industrial waste treatment plants. Sewage and Industrial Wastes 29: 134-142.

Peters, B. G. 1930a. Biological investigation of sewage. Jour. Helminth. 8: 133-164.

-----. 1930b. Some nematodes met within a biological investigation of sewage. Jour. Helminth. 8: 165-184.

Renn, C. E. 1956. Biophysical processes in slime film and floc formation. pp. 116-120 in J. McCabe and W. W. Eckenfelder, Biological treatment of sewage and industrial wastes, Vol. 1. Aerobic Oxidation. Reinhold Publ. Co. New York.

Reynoldson, T. B. 1939. Enchytraeid worms and the bacteria bed method of sewage treatment. Ann. Appl. Biol. 26: 138-164.

Stanbridge, H. H. 1954. The development of biological filtration. Water and Sanitary Engineer 4: 297-300, 326.

-----. 1955. The development of biological filtration. Water and Sanitary Engineer 5: 252-255.

Standard Methods for the Examination of Water, Sewage and Industrial Wastes. 1955. 10th ed., pp. xix, 522. Amer. Pub. Health Assn., Inc., New York.

Tomlinson, T. G. 1941. The treatment of settled sewage in percolating filters in series with periodic change in the order of the filters. II. Biological investigations. Jour. and Proc. Inst. Sew. Purif.

-----. 1946. Growth and distribution of film in precolating filters treating sewage by single and alternating double filtration. Journ. and Proc. Inst. Sewage Purif.

Usinger, R. L. and W. R. Kellen. 1955. The Role of insects in sewage disposal beds. Hilgardia 23: 263-321.

Whipple, G. C., G. M. Fair and M. C. Whipple. 1947. The Microscopy of Drinking Water, pp. xix, 585. John Wiley and Sons, Inc., New York.

Wilson, J. N. 1943. Biological investigations of high-rate trickling filters, In G. Walton, High daily rate trickling filter performance. Upper Mississippi River Basin Sanitation Agreement. Bull. pp. 111-136, 1943.

-----. 1949. Microbiota of sewage treatment plants and polluted streams, In Limnological Aspects of water Supply and Waste Disposal. A.A.A.S., pp. 1-15.

Reproduced With Permission From, and Copyrighted By:
AMERICAN JOURNAL OF PUBLIC HEALTH
50(1960): 215-219

SELECTION AND ADAPTATION OF MICROORGANISMS IN WASTE TREATMENT

Paul W. Kabler, M.D., Ph.D., M.P.H., F.A.P.H.A.

Microbial Metabolism is the basic mechanism in the natural purification of polluted waters. Purification of such waters by engineered biological treatment processes, i.e., activated sludge, trickling filters, oxidation ponds, etc.,[1] is, in this respect, not essentially different from natural stream self purification. Polluted waters in this presentation include sewage and industrial wastes and surface waters receiving these wastes.

It is recognized[2] that biological treatment is neither feasible nor advisable for some industrial wastes containing inhibitory or toxic substances. This category includes inorganic wastes such as those from metal-pickling and plating processes. Even these wastes are sometimes discharged into municipal sewers and into streams and affect the biological processes therein. Often industrial wastes contain mixtures of inorganic and organic compounds, either of which may exert biologically inhibitive effects. Many such wastes contain synthetic compounds not commonly found in nature, in animal or plant residues. The problems of tolerance levels and of microbial adaptation, synergisms, and antagonisms may become of paramount importance.

The various aerobic biological treatment processes[3] operate on the same fundamental principles and differ primarily by their methods of oxygen transfer. Microorganisms which inhabit these systems include bacteria, fungi, algae, protozoa, rotifers, and higher forms. In the anaerobic systems somewhat less diversity of forms is found; however, the processes embrace the combined activities of a large variety of species. All the treatment processes, either aerobic or anaerobic, as well as the natural self purification of streams, lakes, and impoundments, comprise the aggregate activities of a community of microorganisms acting simultaneously or sequentially and utilizing definitive metabolic patterns and enzymatic pathways.

The transformations of carbon compounds by microorganisms have been excellently reviewed by Nickerson.[4] The common pathways of oxidations for carbohydrates, alcohols, acids, fats, waxes, and aromatic ring compounds are clearly shown. Not all of the enzyme systems described occur in every species of microorganism. It is for this reason that we find mixed populations effectively degrading wastes and a population of which the composition changes in accord with the changing constituents of the polluted environment. Microbial cells may carry on many series of interlocking reactions where the product of one enzyme reaction may be the substrate for several other enzymes, and any modification of even one reaction will produce modifications in diverse reactions, many steps removed from the initial position.

The biological transformations of compounds containing nitrogen[5] and sulfur[6] have also been extensively studied. The degradation of nitrogen compounds will invariably be accomplished by hydrolysis of proteins and other complex nitrogen molecules, and will result in the eventual decomposition of the lower molecular weight residues. The degradation of these compounds will vary with aerobic or anaerobic conditions, and rigid control is necessary to avoid anaerobic conditions that result in the production of noxious materials. Diverse microorganisms participate in degradation of sulfur compounds, many of which command attention as they produce compounds having objectionable odors or causing corrosion of industrial equipment. Two principal sulfur products are formed, sulfate under aerobic conditions and sulfide under anaerobic conditions.

The ultimate goal of natural or of biologically-controlled purification is to transform any pollutional material into innoxious components. To achieve this end it is sometimes necessary to develop specialized populations or to provide unusual environments. Such specialized populations (seeds) have been described for the treatment of a variety of naturally occurring and synthetic materials. This development is frequently termed "acclimatization," but more properly should be considered selective growth and adaptation of organisms resident in the environment.

Among the large number of microbial species extant, some species will be relatively well adapted to any environment encountered in polluted water, stream sediments, or in artificial treatment processes. And since the species of microorganisms involved in such processes are ubiquitous,[7] we can expect a suitable organism to be present when a specific environment arises. Any sample of soil or polluted stream bottom contains countless kinds of microenvironments, some high in nutrient and some low, some aerobic and some anaerobic, and each has its characteristic biota. Furthermore, microbes can with some exceptions lie dormant for long periods when their environment is unfavorable. This characteristic of being universally present and yet yielding the environment to the "best fitted" species seems to explain a large portion of microbial adaptations, as well as to account for the well recognized usefulness of stream muds or sludges as starters in treatment plants.

Scientific and industrial activities are changing the environment by the addition of chemical compounds which have not been components of soil or aqueous environments in the past, and organisms are confronted with new chemical entities in concentrations and under conditions not previously known. For organisms to appear that can utilize these new chemicals, it is necessary that large numbers of the wild type be challenged and that selective processes prevail. With some of the newer organics, such selections have not as yet been completely successful; however, there appears to be no reason why suitable organisms should not be found if the search is sufficiently diligent.

All living organisms from microbe to man have become adapted to their habitat, i.e., to its energy supplies, temperature, and other aspects, and all react to multiple factors of this environment. Therefore, in considering microbial interactions in natural habitates or in conventional treatment processes--in soil, trickling filter, or sludge digestion tank--we must realize that the microbes live there in populations of billions, and that they intermingle on a scale that is impossible for higher plants or animals. Changes in food supply, be it domestic sewage, resistant organic material, or some chemical compound developed in the future, will directly support only that portion or portions of the population that can utilize the food available. The medium and conditions of growth usually select specific individuals from the inoculum (seed) most fit to survive in the new environment. Usually these grow better after a period of training through repeated generations in the new menstrum, which means the selection of species of a "superior" growth type. Because this superior growth is observed only if the organisms that possess it pass it on to their descendants, often the resultant population will be the progeny of one or a few individuals of the original inoculum. In this way selective populations are developed, as has been so adequately demonstrated by special isolation technics[8-10] and the adaption of treatment processes to chemical compounds.[11-13]

Species selected in this fashion must make further adjustments in order to survive and to reproduce. These adjustments are frequently termed adaptation and are related to microbial ability to acquire or to activate enzymes for the use of compounds not initally available to the organism as food. Such adaptations are not usually retained by the organism when the compound is removed from its environment. Transfer of these adaptive qualities in active enzyme systems from one generation to the next apparently does not involve changes in the genetic pattern, but rather appears to depend on the necessity for the organism to use the particular characteristic in maintaining life. Some of these modifications are direct, as when a culture of bacteria is exposed to penicillin. Each cell of the population starts to produce the enzyme penicillinase which destroys the penicillin. There is no selection; each cell has the genetic ability to produce the enzyme but does so only when the material is present. The elaboration of an enzyme when it is useful and the ability to grow without it when it is of no value appears to be the basis for much of the on-the-spot adaptation of microbes. One well known example is the enzyme production in some coliform bacteria that metabolize lactose. This enzyme may appear only after the culture has been incubated several days. The environment changes the organism but only within the limits of its genetic make-up. These modifications are termed induced[14] or adaptive enzyme formation.

There is another mechanism whereby superior strains may arise as a result of a sudden change in the genetic make-up, which is passed on at cell division. These changes in microorganisms are called mutations and supposedly occur in any of the numerous "genes" present in the cell, at an average rate of about once in every ten million divisions[15,16] per gene. This mutation rate of each gene has been determined naturally by evolutionary challenge, since if mutation occurred too seldom, the organism could not successfully compete in a changing environment, and if too often, too many individuals would be lost in wasteful modifications.

Even with one new mutant occurring per ten million cells at every generation, it would seem that after a few generations nearly all the organisms would be mutant in one or more characteristics. However, in actual experience, cultures transferred under constant conditions remain relatively stable over long periods, partly because many of the mutants are slower growers or otherwise not as well fitted to the environment as the parent organism. When new mutants appear which are superior in growth characteristics, they overgrow and eliminate the parental type together with existing intermediate or stray mutants, until the new in turn are eliminated by the same process.

The mutation rate can be modified by environmental conditions, such as ultraviolet light, radiations, certain organic and inorganic materials, metabolic products, etc. Many of these induced mutants are less able to compete in the environment than the parent types and are lost, so that an investigator may or may not be successful in recovering a predetermined desirable mutant by such methods of treatment.

Another possible method by which bacterial species may arise that are highly efficient in metabolizing complex substrates is that of gene recombination as described by Tatum and Lederberg.[17] In recombination, the resultant progeny of two parent strains carry the ability to metabolize a complex menstrum for which each parent possesses only a fractional ability, i.e., the offspring exhibit the combined characteristics of both parents. If one parent is capable of metabolizing fraction A of a complex and the other parent has the ability to utilize fraction B, the resulting offspring may be able to utilize the whole AB complex. When one considers the infinite number of microbial species present in natural habitats, it does not seem unlikely that some will be closely related and can produce desirable progeny that can accomplish the purification of highly complex and resistant materials.

In transformation, a particular characteristic is transferred from a donor strain of cells to a recipient strain which had not previously exhibited it. This transfer can be accomplished artificially by bringing desoxyribonucleic (DNA) acid of the donor cell nuclear apparatus into contact with the recipient bacteria, which incorporate the donated DNA into their cell substance. Acquisition of the new DNA fraction imparts to the recipient cells the characteristic originally carried by the donor strain. In mixed cultures of related microorganisms, this transfer by DNA can occur naturally when some of the donor cells become aged and break up, thus liberating the cellular components into the growth menstrum.

In transduction, the transfer of a characteristic is again accomplished by transfer of donor cell DNA to recipient cells. However, the actual mechanism of transfer is through phage particles acting as vectors. For this mechanism to function, it is necessary that both donor and recipient strains be susceptible to the phage. Phages have differing ranges of infectivity, but most are able to infect only closely related microorganisms. Therefore, transduction is likely only between closely related strains.

It seems certain that the selection and adaptation of a competent microbiota in waste treatment processes is a highly complex chain of events. It is likely that the selection from natural reservoirs of those microbial species capable of metabolizing the waste components, together with activation of latent enzymes, accounts for a large part of the adjustment necessary to propagate a useful population. Natural or induced mutations may be instrumental in modifications enabling organisms to metabolize materials such as "synthetics" which are not normally present in natural environments.

In recent experience, it has been possible to develop satisfactory microbial populations to treat most domestic and industrial organic wastes. Whether populations will be developed that can effectively degrade resistant materials such as alkyl benzene sulfonate and lignin must remain speculative at this time. The processes involved in such a chain of events will likely include selection of the "most fit to survive" progenitors, activation of certain latent enzyme systems, and probably genetic changes. Chemical pretreatment or conditioning of resistant materials for biological breakdown may be a possibility.

SUMMARY

Artificial and natural purification processes are accomplished by the combined metabolic activities of a number of microbial species and variants. The active members of a particular system are dependent on the composition of the substratum, i.e., its organic and inorganic components, pH, temperature and have been selected and adapted from the infinite number of microorganisms and microenvironments extant in nature, resident in soil, bottom muds, composts, and other materials.

Selection is accomplished by fortuitous matching of potentially competent microorganisms of the habitat and appropriate available waste materials.

Further adaptations are accomplished by producing necessary new enzymes either through activation of latent characteristics or through genetic changes that arise spontaneously or by stimulation from environmental factors.

Mutations may produce variants either more or less suited to the environment. Only the "more fitted" progeny are likely to survive.

The parts played by recombination, transformation, and transduction in the selection of the microbial biota in waste treatment processes may be im-

portant but cannot at present be fully evaluated.

REFERENCES

1. Ettinger, M. B. Universal Factors in Aerobic Biological Purification. Sew. & Ind. Wastes 30: 14. 1958.

2. Heukelekian, H. Purifying Chemically Polluted Water. Basic Biological Principles. Ind. & Eng. Chem. 48: 1403. 1956.

3. McKinney, R. E. Microbiological Process Report. Activity of Microorganisms in Organic Waste Disposal . II. Aerobic Processes. Applied Microbiol. 5: 167. 1957.

4. Nickerson, W. J. Purifying Chemically Polluted Water. Transformations of Carbon Compounds by Microorganisms. Ind. & Eng. Chem. 48:1411. 1956.

5. Delwicke, C. C. Biological Transformations of Nitrogen Compounds. Ibid. p. 1421.

6. Starkey, R. L. Transformations of Sulfur by Microorganisms. Ibid. p. 1429.

7. Wyss, O. Microbial Adaptation. Ibid. p. 1404.

8. Lockhead, A. G., and Chase, F. E. Qualitative Studies of Soil Microorganisms. V. Nutritional Requirements of the Predominant Bacterial Flora. Soil Sci. 55: 185. 1943.

9. Waksman, S. A. Principles of Soil Microbiology. (2nd ed.). Baltimore, Md.: William & Wilkins, 1932.

10. Butterfield, C. T. Studies on Sewage Purification. II. A Zooglea-forming Bacterium Isolated from Activated Sludge. Pub. Health Rep. 50: 671. 1935.

11. Harlow, I. F.; Powers, T. J.; and Ehlers, B. B. The Phenolic Waste Treatment of the Dow Chemical Company. Sew. Works J. 24:1043. 1938.

12. Hamdy, M. K.; Sherrer, E. L. ; Weiser, H. H.; and Sheets, W. D. Microbiological Factors in the Treatment of Phenolic Wastes. Applied Microbiol. 2:143. 1954.

13. McKinney, R. E.; Tomlinson, H. D.; and Wilcox, R. L. Metabolism of Aromatic Compounds by Activated Sludge. Sew. & Ind. Wastes 28:547,1956.

14. Spiegelman, S., and Campbell, A. M. "The Significance of Induced Enzyme Formation," in Currents in Biochemical Research. New York, N. Y.: Interscience Publications. 1956.

15. Demerec, M. "Genetic Aspects of Drug Resistance. " in Drug Resistance in Microorganisms. Boston, Mass.: Little, Brown, 1958, p. 51.

16. Braun, W. Bacterial Genetics. Philadelphia Pa.: Saunders. 1953.

17. Tatum, E. L., and Lederberg, J. Gene Recombination in the Bacterium Escherichia coli. J. Bact. 53: 673. 1947.

18. Hotchkiss, R. D. Transfer of Penicillin Resistance in Pneumococci by the Desoxyribonucleate Derived from Resistant Cultures. Cold Spring Harbor Symposium on Quantitative Biology 16:457, 1951.

19. Zinder, N. D., and Lederberg, J. Genetic Exchange in Salmonella. J. Bact. 64: 679, 1952.

Dr. Kabler is chief of microbiology, Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio.

This paper was presented before a joint session of the Conference of State Sanitary Engineers and the Conference of Municipal Public Health Engineers and the Engineering and Sanitation Sections of the American Public Health Association at the Eighty-Sixth Annual Meeting in St. Louis, Mo., October 29, 1958.

THE FOLLOWING PAGES ARE DUPLICATES OF ILLUSTRATIONS APPEARING ELSEWHERE IN THIS REPORT. THEY HAVE BEEN REPRODUCED HERE BY A DIFFERENT METHOD TO PROVIDE BETTER DETAIL